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Gulf Breeze, FL 32561

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FOREWORD

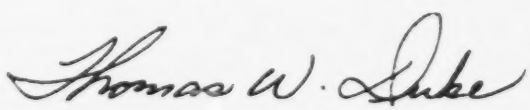
In December 1970, as Director of a U.S. Fish and Wildlife Service Laboratory on Sabine Island, I had the gratifying experience of helping integrate the Laboratory's research program into the newly created Environmental Protection Agency (EPA). It was a time of change, marking the birth of the first governmental organization charged with examining pollution problems in terms of the total inter-related environment.

September 1979 also represents a time of change for me. I have requested and received reassignment to a scientific position at the Environmental Research Laboratory, Gulf Breeze (ERL,GB). This assignment will provide me the opportunity to work more closely on scientific aspects of regulatory actions. I will begin the reassignment by working a year at Texas University's Institute of Marine Science in Port Aransas, Texas, under the Intergovernmental Personnel Act. While at the Institute, I plan to research the impact of drilling mud on an indigenous benthic community and to become involved in their academic program. The assignment at Port Aransas will provide a transition from administrative aspects of a regulatory laboratory to more scientific aspects. My experiences during the past few years have convinced me more than ever that environmental regulations must be based on a strong scientific data base and that development and interpretation of the base requires our collective best effort.

In retrospect, I have had a unique opportunity of working with many other scientists (from ERL,GB, EPA, Federal, State and local government agencies, and educational institutions) in designing research programs and recommending standards for a healthy, productive environment.

Our research has provided a basis for many EPA regulatory decisions related to hazards posed by toxicants to estuarine and marine resources. It has also demonstrated that many important questions remain to be answered if scientists are fully able to comprehend the total effect of pollutant effects on marine animals and their environment. Some investigations have uncovered uncertainties that must be resolved. It is now clear that new initiatives must be forthcoming, and new talents must be recruited.

I look forward to the experiences to come and hope to gain new insights to problems related to the development of a pertinent data base for environmental regulations. However, I say goodbye to the dedicated staff at ERL,GB and to the supporters of our research efforts with deep regret. I have always felt the staff of this Laboratory was supportive to me as I attempted to direct research efforts. I am especially appreciative of the understanding and support the staff has given me and my family during the last few months. I am optimistic about the future of this Laboratory and being a part of it.



Thomas W. Duke
Director
Environmental Research Laboratory
Gulf Breeze, Florida

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16. ABSTRACT

This report reviews aquatic research programs conducted or managed by the Environmental Research Laboratory, Gulf Breeze, Florida, for the Office of Research and Development, U.S. Environmental Protection Agency in 1978 and 1979. The research program examines the impact of pesticides and other organic compounds on marine species and communities, and seeks to develop new methodology for determining ecological hazards of chemicals under conditions simulating the natural environment. Projects are outlined in the areas of: Exposure Assessment, Effects Assessment, Chlorination Studies, Offshore Oil Drilling, Environmental Pathobiology, and the Chesapeake Bay Program.

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NOTE: **This report is for informational purposes only. All data and conclusions must be considered provisional. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.**

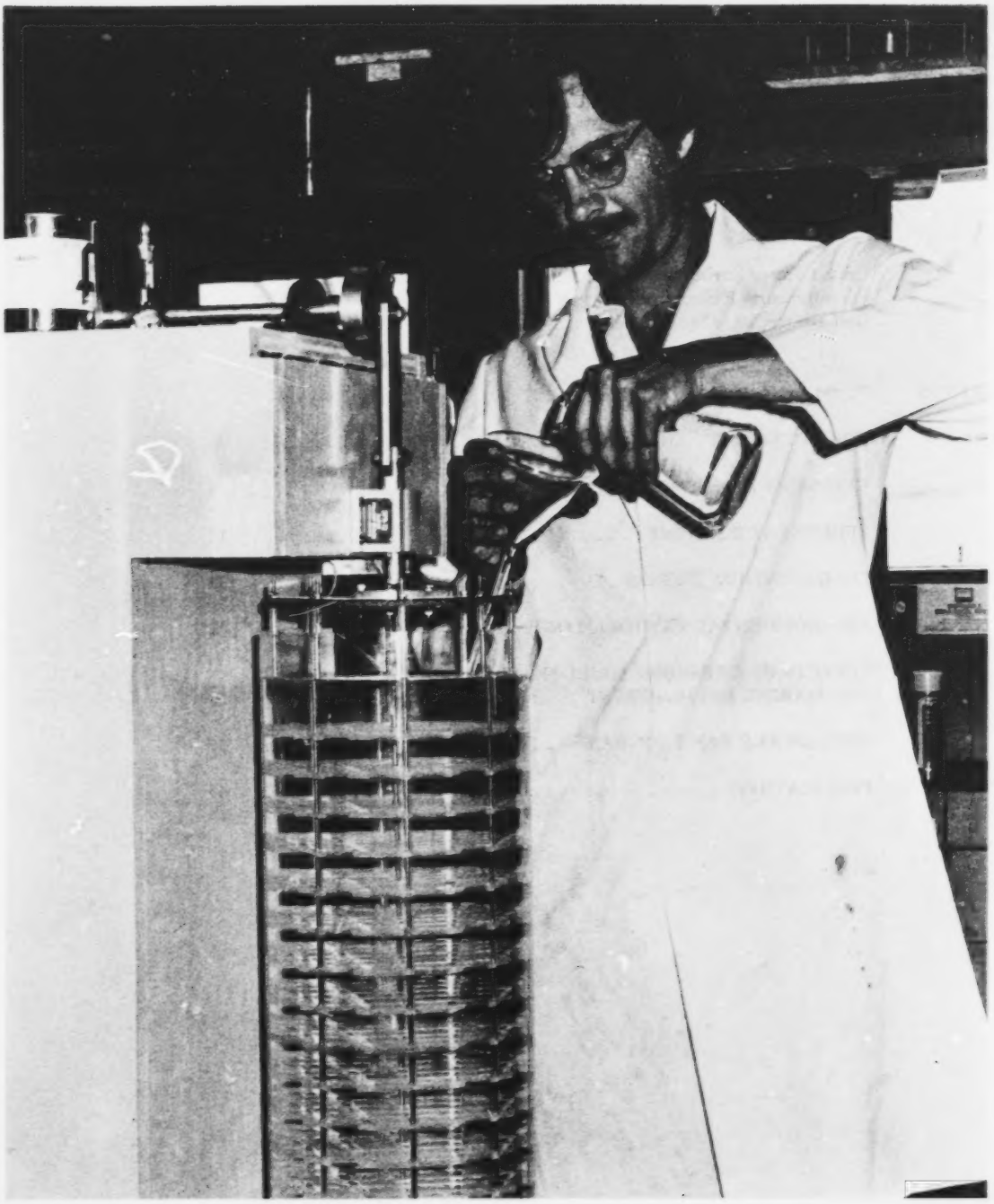

Figure 1. *Environmental Scientist J.P. Connolly adds an aluminum powder solution to illuminate the flow field in a turbulence generation apparatus used to investigate the effects of sediment resuspension on the environmental fate of toxic substances.*

EXPOSURE ASSESSMENT

A dramatic increase in the use and production of chemicals in the United States has prompted the development and manufacture of many new compounds that have subtle, yet-to-be determined ecological consequences.

EPA legislative mandates require a reliable data base for the evaluation of human and environmental risks posed by toxic substances. In 1978, ERL,GB researchers continued to work on the development of new techniques and methodology for Exposure Assessment applicable to the development of criteria for the control of toxic substances and the registration or reregistration of pesticides.

A primary objective of the Exposure Assessment effort was to develop a simple screening tool that can be used in the preliminary characterization of the fate of pollutants in estuaries. Data from the preliminary tests will form the basis for subsequent toxicological tests and more complex determinations of the transfer and fate of pollutants in the marine or estuarine environment.

Fate Studies

A.W. BOURQUIN, Research Microbiologist;
H.P. PRITCHARD, Environmental Scientist

ERL,GB researchers have developed a three-level (tier) test to predict the fate of toxic organics in estuarine systems. Procedures are based on tier-testing and allow extrapolation to real-life aquatic settings. The system eventually will be integrated into a larger Laboratory Hazard Assessment Program that is now under development.

The central concepts for the tier-test system are:

Level I--rapid screening tests that provide preliminary information on detoxification, biodegradation, and transformation processes. These screening tests provide information for orientation toward more elaborate testing for both fate and toxicity effects.

Tests use actual media, sediment, and water, and require no radiolabeled chemicals. Sterile systems are employed to determine the chemical hydrolysis contribution to the transformation process. Loss of toxicity will be assayed with mysid shrimp and a benthic amphipod.

Level II--a laboratory sediment-core test system capable of generating "range finding" data that integrates fate processes under the complexities of a natural estuarine environment. Testing protocol includes relative estimates of routes, rates, and the extent of fate processes relative to standard test chemicals.

Level III--laboratory microcosm tests that simulate natural estuarine conditions and quantitatively evaluate rates, extent, and capabilities of degradative processes on toxic organics leading to predictions of exposure concentrations.

Fate Study (Dimilin®)

A.W. BOURQUIN, Research Microbiologist;
H.P. PRITCHARD, Environmental Scientist

Of the new type of insecticides that evoke toxicity through interference with the formation of the exoskeleton, Dimilin® (diflubenzuron) is probably the most widely used. In order to properly assess Dimilin's potential toxic effects in marine environments, a fate study has been conducted by researchers at ERL,GB to compare and contrast results derived from soil studies conducted by other scientists with the insecticide.

In screening tests, Dimilin was shown to have a long P (octanol/water partition coefficient) of 3.02, a sediment/water partition coefficient of 350, and no substantial volatility. Studies in a static core sediment/water laboratory test system (Eco-core) and in a continuous-flow microcosm have revealed that Dimilin readily hydrolyzes into *p*-chlorophenyl urea and diflurobenzoic acid. In the static Eco-core, 90% of the original Dimilin hydrolyzed after 24 days, indicating a half-life of 17 days.

The hydrolysis products were shown to be non-toxic to mysid shrimp and did not degrade even after long-term incubation. In similar soil studies reported in the literature, shorter half-lives were observed and the hydrolysis products were readily biodegraded. Only very small amounts of Dimilin became bound to the sediment used in these systems. Thus, there appears to be definite differences between fate processes in estuarine and terrestrial environments.

Studies in the continuous-flow microcosm showed similar results. Tests increasing the number of active growth vessels or lengthening the exposure to sediment revealed that Dimilin hydrolysis was largely biological and sediment-mediated (Fig. 2). Since these studies involve naturally derived sediment/water systems, results probably reflect similar processes that occur in estuarine salt-marsh environments. Dimilin appears to break down about two times slower than methyl parathion under similar laboratory conditions.

Sediment-core (Eco-core)

A.W. BOURQUIN, Research Microbiologist;
H.P. PRITCHARD, Environmental Scientist

ERL,GB researchers have developed a sediment-core fate test, known as Eco-core, that has been proposed as a standard degradation test system for marine environments. The system involves analytical chemical tracing of the fate of a radiolabeled pesticide or toxic organic added to an

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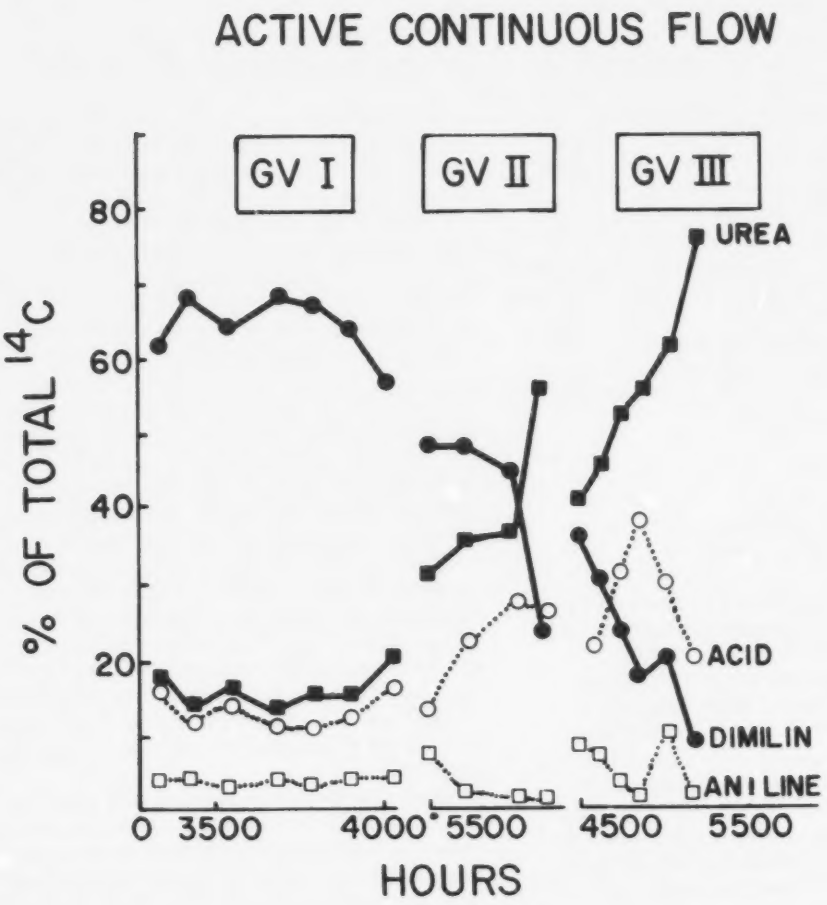

Figure 2. The fate of Dimilin and its hydrolysis products in continuous-flow microcosms shown in three consecutive growth vessels (GV I, II, III). (●) Dimilin, (■) parachlorophenyl urea, (○) 2,4-difluorobenzoic acid, (□) parachloroaniline.

undisturbed sediment/water core. The Eco-core system offers these advantages:

(1) It is simple, inexpensive, and easily assembled.

(2) Replication with the Eco-core is excellent.

(3) Many Eco-cores can be set up at once to permit the simultaneous testing of several environmental parameters.

(4) Experimental protocols allow the fate of a toxicant to be followed with time, and the distribution of the toxicant in water and sediment (total budget analysis) to be determined.

(5) Results from an Eco-core test reflect an integrated picture of all the fate processes occurring in the sediment/water core. With the proper controls, however, rates and extents of chemical hydrolysis, sediment binding, microbial degradation, and volatilization can be individually assessed.

(6) Because a sediment water core is employed, the sediment/water interface is relatively undisturbed. Natural environmental conditions and complexities (including anaerobic conditions in the sediment) are maintained, thus giving validity to data generated.

(7) Simple operations and analyses permit a large number of compounds to be tested; a sufficient data base can be generated to provide fate assessment based on relative rankings with reference compounds.

Figure 3 presents the results of an experimental series of Eco-cores using sediment from Range Point saltmarsh and three pesticides: methyl parathion, pentachlorophenol, and Sevin®. The data qualitatively and quantitatively demonstrate that in estuarine saltmarsh samples, Sevin degrades fater than methyl parathion, which degrades faster than pentachlorophenol. Table 1 gives a total budget analysis from the same experiment. Because of extensive calibration, standardization, and characterization, the Eco-core test system can be applied to fate studies in aquatic systems for many other pesticides and toxic substances.

Mathematical Modeling of Fate and Transport Processes

J.P. CONNOLLY, Environmental Scientist

A new program in mathematical modeling of fate and transport processes in estuarine environments was initiated at ERL,GB in 1978. The program is divided into four categories:

(1) The development of mathematical models of sediment adsorption/desorption processes. Studies focus on the role of organic matter, sediment concentrations, and sediment dispersion on these sorption processes.

(2) Development of mathematical models to describe fate processes studied in laboratory microcosms. Special emphasis was placed initially on continuous-flow microcosms developed at ERL,GB. Rate constants were sought for fate processes (i.e., degradation, hydrolysis, sorption, volatility, photolysis) that simulate natural conditions as much as possible.

(3) Development of mathematical models for predicting exposure concentration in estuarine environments. In 1978, the James River model developed in conjunction with Manhattan College was utilized as an example of a typical East Coast estuary offering extensive background, ecological, hydrodynamical, and physiographical information. Studies with Kepone are being employed for calibration purposes.

(4) Conceptualization of models for hazard assessments in marine ecosystems. This work is designed to integrate acute and chronic LC50's, bioconcentration, behavior, and fate process information generated at the ERL,GB into an assessment of specific pesticides and to identify areas requiring further research (Fig. 1).

Aquatic Bacteria Studies

A.W. BOURQUIN, Research Microbiologist;
P.H. PRITCHARD, Environmental Scientist

Effects of toxicant concentrations on the ability of microorganisms to degrade toxicants were investigated. In 1978, a variety of aquatic bacteria were isolated and grown; low concentrations of phenylacetic acid (a base chemical for the synthesis of certain herbicides) serve as their sole source of carbon and energy. Evidence from other sources indicate that these bacteria may be adapted to growth at such low concentrations because of a unique physiological condition. In the ERL,GB oxygen uptake studies, cells originally isolated with phenylacetic acid (PAA) as the substrate continued to produce the PAA catabolic (constitutive) enzymes even when grown on acetic acid (without PAA in the medium). This response is atypical for most aquatic bacteria obtained by conventional means and seems to correlate with the organism's ability to grow at low concentrations.

Further studies will be undertaken to more fully characterize this constitutive response. If the response is common in bacteria growing at low nutrient concentrations, the chance for the occurrence of certain types of biodegradation processes (cometabolism) for pesticides or toxicants in aquatic systems could be high.

Biodegradation Workshop

The Proceedings of the Workshop on Microbial Degradation of Pollutants in Marine Environments, hosted April 9-14 by ERL,GB, were published in June 1979 in the EPA Ecological Research Reporting Series (EPA-600/9-79-012).

The workshop was sponsored by the EPA Office of Research and Development, Office of Toxic Substances, and Office of Pesticide Programs, and Georgia State University to evaluate biodegradation studies in aquatic environments, to develop protocols for methodology, and to define research needs and experimental limitation in this field.

Government agencies, universities, and industrialists were represented in workshop sessions concerned with fate studies, regulation, or production of potential aquatic pollutants. ERL,GB Microbiologists A.W. Bourquin and P.H. Pritchard co-chaired the workshop.

Determination of the Environmental Impact of Substitutable Chemical Pesticides in Agriculturally Affected Wetlands

J.W. DAY, Jr., S.P. MEYERS, and R.P. GAMBRELL, Principal Investigators; EPA Grant R804976, Louisiana State University, Baton Rouge, LA; F.G. WILKES, Project Officer

This research will attempt to determine: (1) the fate of pesticides, such as Guthion®, in brackish wetlands; correlating results with past productivity, nutrient flows, application rates, and runoff patterns; (2) the fate and effects of toxic substances applied to test plots; (3) the persistence of pesticides under varying conditions; and (4)

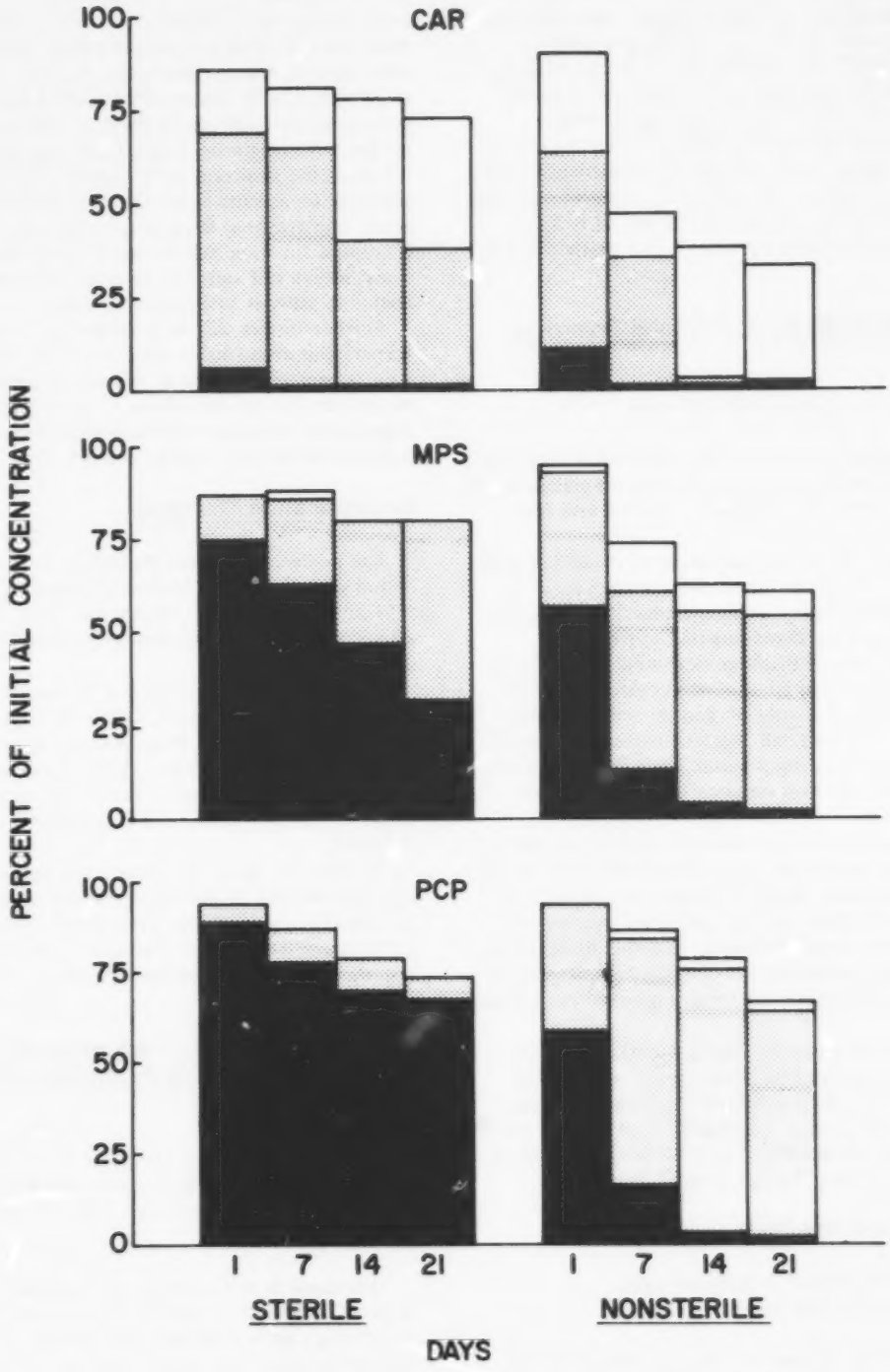

Figure 3. Eco-core microcosm from Range Point, FL, salt marsh. The total ^{14}C in the water column ☐, the ethyl acetate-extractable portion ▩, and the residual parent compound ▩, are shown as percentages of initial concentrations.

TABLE 1a. MASS BALANCE OF CARBON-14 IN SALT-MARSH MICROCOSMS RECEIVING ^{14}C-PENTACHLOROPHENOL (% OF CARBON-14 INITIALLY ADDED)

	Day 1		Day 7		Day 14		Day 21	
	*S	**NS	S	NS	S	NS	S	NS
$^{14}CO_2$	0	0	0	0	0	0	0	3
Water	95	94	84	87	81	82	82	79
Sediment								
Extractable	5	6	15	7	9	16	12	13
Nonextractable	1	1	1	2	5	4	5	1
Total Recovery	101	101	100	96	95	102	99	96

TABLE 1b. MASS BALANCE OF CARBON-14 IN SALT-MARSH MICROCOSMS RECEIVING ^{14}C-METHYL PARATHION (% OF CARBON-14 INITIALLY ADDED)

	Day 1		Day 7		Day 14		Day 21	
	*S	**NS	S	NS	S	NS	S	NS
$^{14}CO_2$	0	2	0	4	0	4	0	8
Water	82	90	88	74	76	73	76	70
Sediment								
Extractable	11	5	11	5	13	6	4	8
Nonextractable	3	3	2	7	4	8	4	8
Total Recovery	96	100	101	90	93	91	84	94

TABLE 1c. MASS BALANCE OF CARBON-14 IN DISPOSAL POND MICROCOSMS RECEIVING ^{14}C-CARBARYL (% OF CARBON-14 INITIALLY ADDED)

	Day 1		Day 7		Day 14		Day 21	
	*S	**NS	S	NS	S	NS	S	NS
$^{14}CO_2$	0	1	0	30	0	26	***ND	20
Water	89	80	75	29	74	19	ND	18
Sediment								
Extractable	2	2	9	7	15	4	ND	5
Nonextractable	3	8	8	24	6	42	ND	43
Total Recovery	94	91	92	90	95	91	ND	86

*S = Sterile
**NS = Nonsterile
***ND = Not Determined

new techniques for correlating laboratory and field methods (Fig. 4).

The physical/chemical studies focus on the effects of different Eh and pH conditions on pesticide stability. Organisms isolated from batch cultures are tested for their ability to degrade the pesticide in pure culture. Another microbiological study concerns the effect of pesticide addition in a chemostat on microbial populations from field samples. Effects of pesticides on nitrifying bacteria are under investigation.

The effect of redox potential on the levels of Guthion in swamp soil at pH 7 is presented in Fig. 5. Guthion essentially disappeared from the most oxidized suspension (+450 mv) in 6 days. At +250 mv, approximately 6 parts per million (ppm) Guthion remained at 6 days, disappearing by day 12. Moderate and strong reduction conditions (+5, −150 mv) decreased the rate of Guthion loss compared to better-oxidized treatments; some Guthion (approximately 0.5 ppm) was recovered at 20 days.

Several microorganisms in soil-water microcosms that exhibit an ability to degrade Guthion have been isolated; studies on rates, utilization, and degradation of Guthion are in progress. In addition, several organisms have demonstrated resistance to the pesticide. No significant change in growth rate as a result of pesticide exposure has been found.

Guthion was found to be very toxic to benthic invertebrates in field studies. At concentrations above 1 ppm, the community population was reduced at 95% (many species were reduced by 100%). System alteration occurred even at 0.01 ppm.

The population of benthic communities was reduced by 84% as long as 26 days after exposure. The complete recovery time of swamp communities has not yet been determined; oligocheates and tubellarians appear to recover first and to be affected the least.

Fate and Effects of Atrazine in Salt-Marsh Ecosystems

D.E. DAVIS, Principal Investigator; EPA Grant R803835, Auburn University, Auburn, AL;
F.G. WILKES, Project Officer

Effects of the herbicide, atrazine, on the marshgrass (*Spartina alternaflora*), fiddler crab, periwinkel snail, mussel, and detritus were determined in model ecosystems. Atrazine stress effects in a natural salt marsh were compared with effects observed in the laboratory.

Atrazine was sprayed at 0.0, 0.5, 5.0, and 50.0 kg/ha on triplicate plots in a salt marsh on Sapelo Island, GA, and on microecosystems built to simulate the salt marsh. Residue levels were determined 3 months after spraying. The top 25 cm of Sapelo soil treated with 50 kg/ha contained approximately 4% of the atrazine applied; the microecosystems contained approximately 3%. Atrazine concentrations in 0 to 1, 1 to 10, and 10 to 25 cm of Sapelo Island soil averaged 1.18, 0.74, and 0.23 ppm, respectively. Levels from the microecosystems were lower. Soils from plots receiving 5.0 or 0.5 kg/ha contained the lowest atrazine concentrations, which were 0 to 0.04 ppm below the top 1-cm layer.

Harvested *Spartina* was divided into: living plants less than 0.5 m, living plants larger than 0.5 m, and dead plants. Residues for these portions of plants from Sapelo Island treated at 50 kg/ha were 2.14, 12.6, and 0.4 ppm, respectively, and for the microecosystems, 16.8, 21.1, and 25.1, respectively. Plants receiving 5 kg/ha had <0.5 ppm atrazine; those receiving 0.5 kg/ha had many values at or near 0.

Periwinkle snails, mussels, and fiddler crabs from microecosystems treated with 50 kg/ha exhibited atrazine concentrations of 7.7, 3.4, and 0.28 ppm, respectively. At Sapelo Island, residue levels in snails and mussels were 0.38 and 0.02 ppm, respectively. There were too few fiddler crabs to assay. None of the animals from the lower treatment rates had greater than 0.2 ppm atrazine, and most had essentially none. Final report for the project will be published in the EPA Ecological Research Series in late 1979.

Fate of 14-C Kepone in Estuarine Microcosms

R.L. GARNAS, Research Chemist

In experiments using static and flowing estuarine microcosms, 14-C Kepone did not degrade under any simulated environmental conditions, but adsorbed rapidly to a variety of sediments.

Adsorption data fitted linear isotherms for a broad range of water concentrations (Fig. 6). The partition coefficient (Kp) of Kepone between sediment and water increased with increasing sediment organic carbon content (O.C.) from quartz sand (O.C. <0.01%) to a salt-marsh sediment (O.C. = 25%).

Field samples (designated with triangles and squares in Fig. 6) from the James River showed a similar concentration dependence on the organic carbon content of sediment. The Kp's for these samples were never below 1000, because the analytical limits of detection were insufficient for lesser concentrations.

Although a decomposed seagrass substrate (ground *Thalassia*) displayed a greater organic carbon content (O.C. = 60%) than a local salt-marsh sediment (O.C. = 25%), the Kp of the seagrass was less. In larger flowing systems, benthic polychaetes (*Arenicola cristata*) accumulated high residues of Kepone, died, and decomposed; however, the Kepone residue associated with this substrate did not desorb as compared to the salt-marsh sediment in the system.

Kepone desorbed readily from salt-marsh sediments and James River sediments and was independent of typical environmental temperature, salinity, and pH ranges.

The discrepancies with the decomposed seagrass Kp and the lack of desorption from decomposed animal tissue would imply that the quality, as well as the quantity, of organic carbon in sediments influence the partitioning of Kepone between sediment and earth.

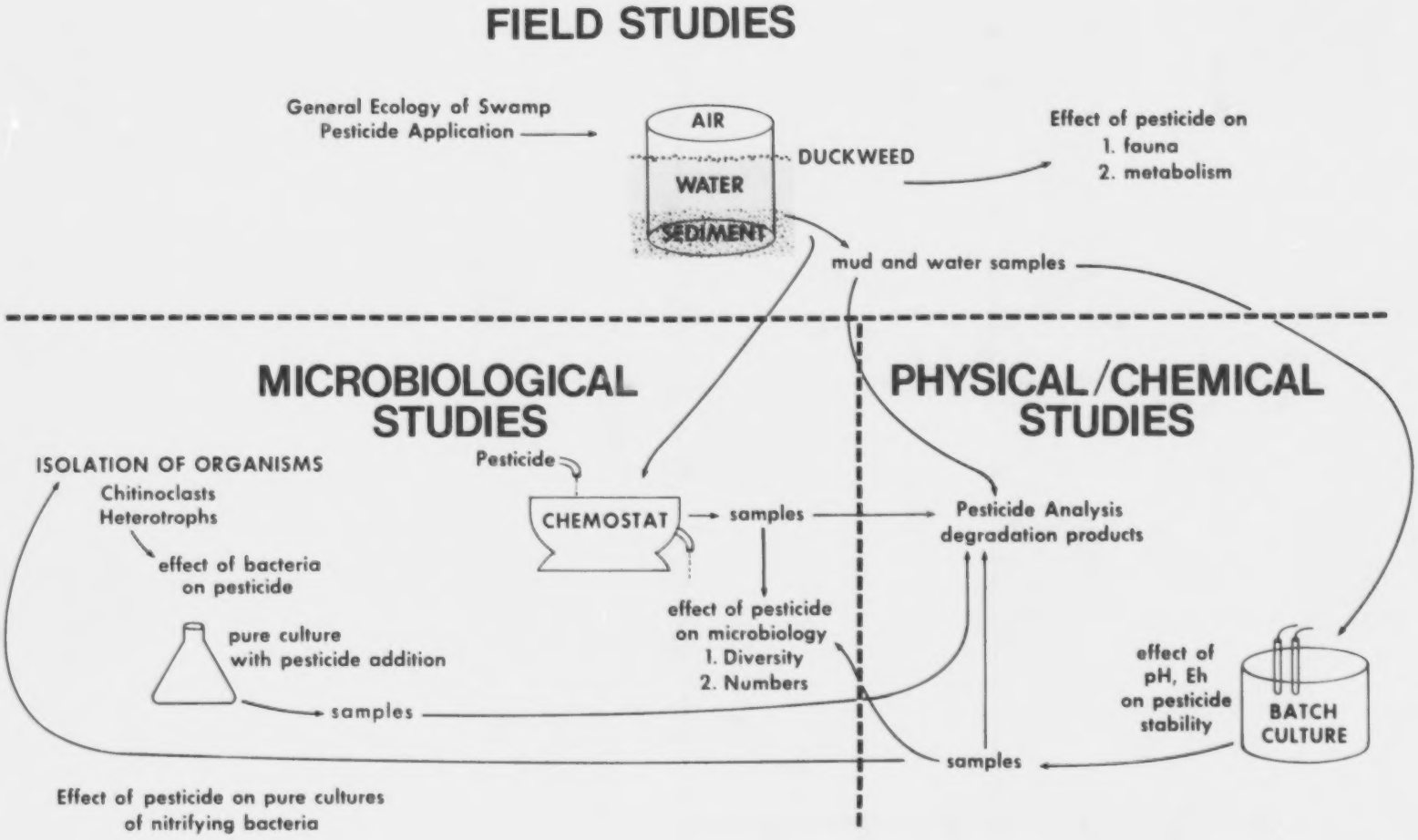

Figure 4. Schematic diagram of the organization of the project.

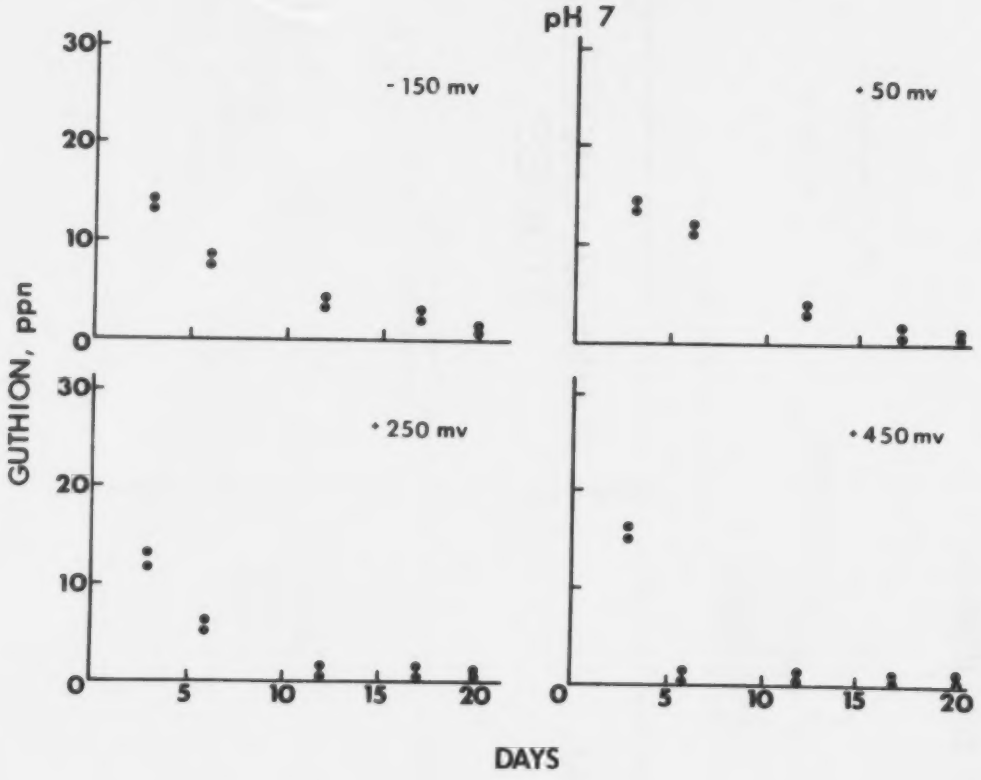

Figure 5. The effect of oxidation-reduction conditions on Guthion loss from swamp soil suspensions maintained at pH 7.0.

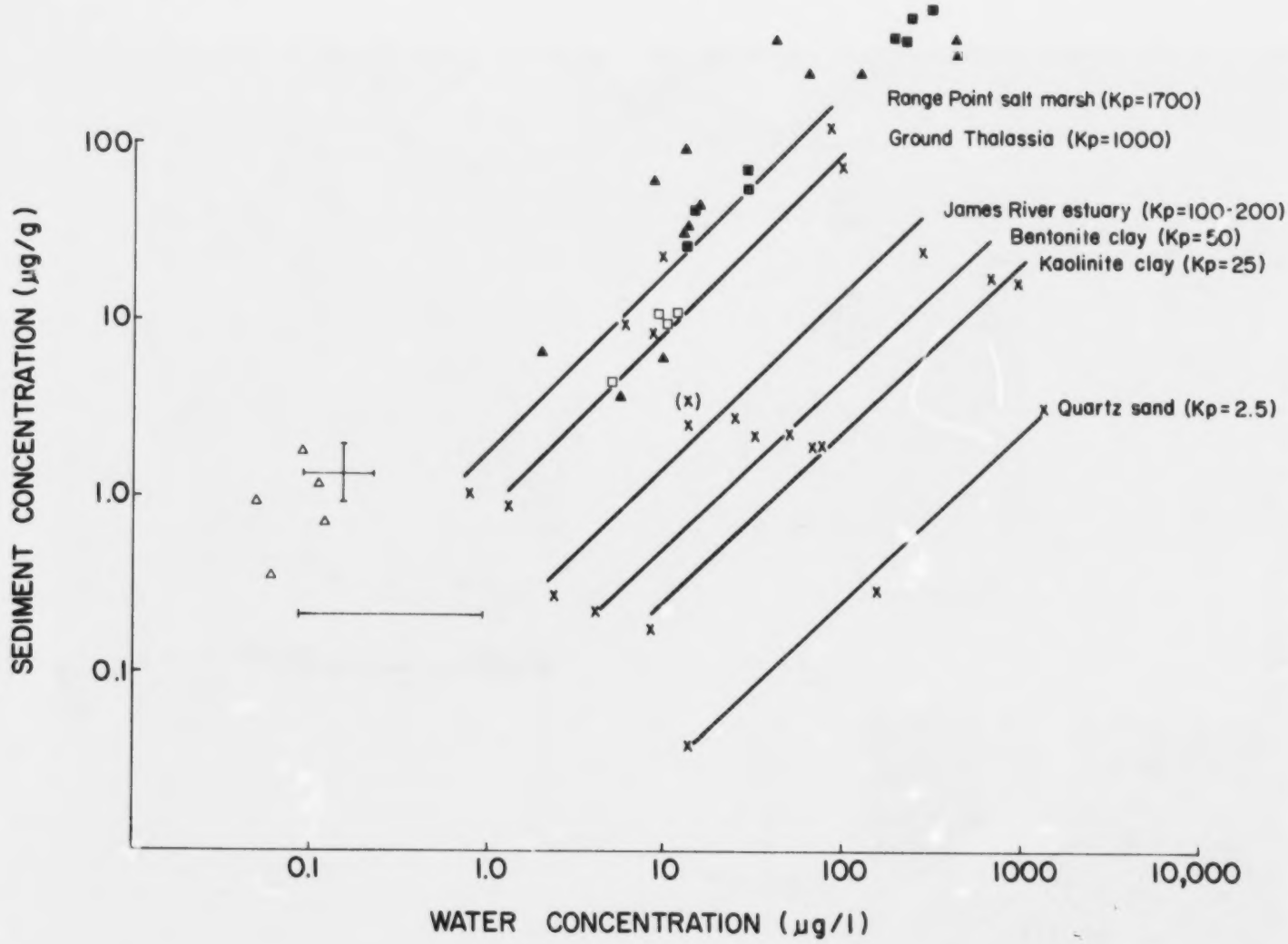

Figure 6. Adsorption isotherms for Kepone in sediments. Triangles and boxes represent data obtained from the James River System.

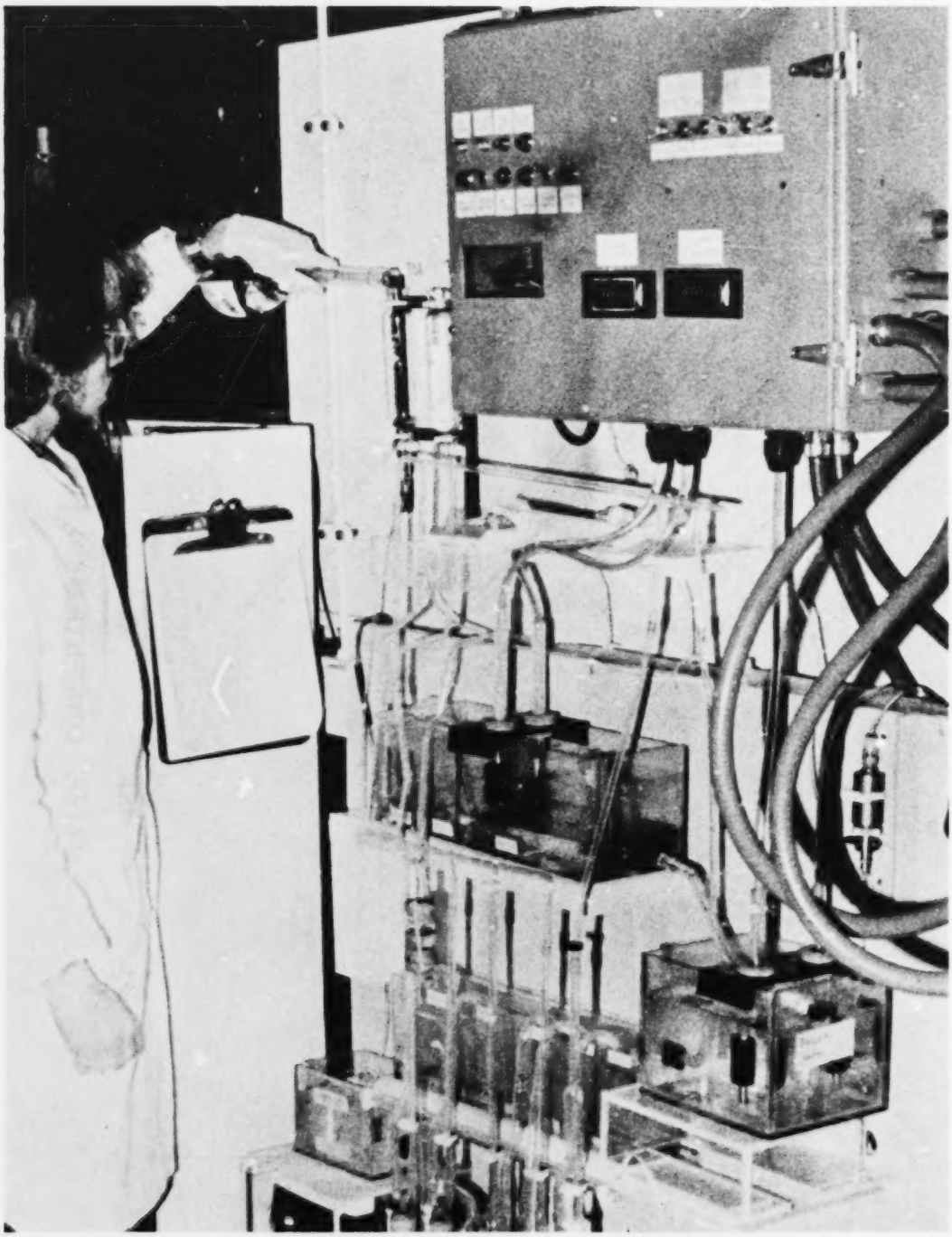

Figure 7. Biological Aide J.V. Wheat fills a carrier control reservoir in a toxicant delivery apparatus for life-cycle toxicity tests using decapod crustaceans.

EFFECTS ASSESSMENT

ERL,GB's Effects Assessment program yields data applicable to EPA requirements for the registration and reregistration of pesticides, development of water quality standards, the issuance of permits for waste disposal in ocean waters, and effluent limitation hearings.

In 1978, Laboratory personnel attempted to develop or improve methods to determine acute (short-term) or chronic (longer-lasting) effects of pesticides and other toxicants on the aquatic environment.

In acute toxicity tests, organisms are exposed to a series of concentrations sufficient to establish (with statistical and chemical precision) the concentrations that reduce survival, growth, or other biological responses.

Chronic effects of pollutants are determined by tests with animals in sensitive life stages (particularly the embryonic and larval stages) or throughout an entire life stage. ERL,GB scientists monitor the effects of toxicants on survival, growth, and reproduction by aquatic species. In addition, effects on the extent and rate of development and on species composition are studied in tests with communities of species exposed to toxicants.

In this reporting period, Laboratory personnel continued to investigate the bioconcentration of pesticides from water by estuarine organisms and the accumulation of these substances in the marine food chain.

The Laboratory's analytical chemistry section in 1978 analyzed approximately 3000 water or tissue samples used in aquatic bioassays for the following pesticides or other organic chemicals: EPN, phorate, DEF®, Trithion®, Guthion®, endosulfan, toxaphene, Aroclor® 1254, Surflo®, pentachlorophenol (PCP), Dimilin®, methyl parathion, trifluralin, and carbaryl.

Acute Toxicity Tests (Dynamic)

S.C. SCHIMMEL, Research Aquatic Biologist;
J.M. PATRICK, Jr., Biological Lab Technician

Data from acute toxicity tests are required by EPA for the development of Water Quality Criteria, evaluations of new, "substitute chemicals," and for pesticide registration, commencing with a Rebuttal Presumption Against Registration (RPAR).

In 1978, acute toxic effects of the defoliant, DEF, and the insecticides, EPN, methyl parathion, phorate, and Trithion were determined in tests using estuarine organisms (Table 2).

In tests with three or more species, the commercially important pink shrimp *(Penaeus duorarum)* was the most sensitive species tested. Its 96-hr LC50 values (concentration estimated to be lethal to 50% of test organisms) were computed to be 1.15 µg/ℓ or less for all five toxicants. Pink shrimp were found to be less sensitive to DEF, but ten times more sensitive to the defoliant than the other estuarine species tested (sheepshead minnows [*Cyprinodon variegatus*], pinfish [*Lagodon rhomboides*], and spot [*Leistomus xanthurus*]).

Bioconcentration Tests

A long-term Trithion® bioconcentration study (15-day uptake, 4-day depuration) in 1978 exposed juvenile fish, spot *(Leiostomus xanthurus)* to 50 µg/ℓ and 5.0 µg/ℓ concentrations of the insecticide. Mortality of spot was observed in the 50 µg/ℓ concentration; therefore, these data were not used. In the 5.0 µg/ℓ concentration (3 µg/ℓ measured concentration), uptake was rapid and 90% of steady-state accumulation was observed in 4 days (Fig. 8). After 15 days, the Trithion delivery was discontinued. Approximately 90% of the accumulation of Trithion at steady-state was depurated in 4 days. The steady-state Bioconcentration Factor (BCF) for Trithion was approximately 600X (based on measured 3.0 µg/ℓ).

Acute Toxicity Tests (Static)

P.W. BORTHWICK, Research Biologist

The lethality of industrial effluents and extracts was investigated in bioassays using the mysid shrimp *(Mysidopsis bahia)* and the sheepshead minnow *(Cyprinodon variegatus)*. Acute, static toxicity tests (96 hr) were conducted with effluent samples from a gunpowder, paper products, and creosote plant (Fig. 9).

Sheepshead minnows survived for 96 hr in all three samples. No fish mortality occurred in concentrations of 100, 56, 32, 18, and 10% effluent, or in controls.

Mysid shrimp, in contrast, appeared to be sensitive to effluents from the gunpowder and paper manufacturing plants. Mysid mortality was significant in 96-hr exposures to 32, 18, 10, 5.6, and 3.2% effluent of paper products plant. In the gunpowder plant effluent, concentrations of 100, 32, and 10% resulted in 100, 30, and 0% mysid mortality. Laboratory findings correlated with simultaneous on-site effluent tests conducted in ERL,GB's Mobile Bioassay Laboratory by Dr. Alan Auwarter.

Chronic Toxicity Test (Fish)

D.J. HANSEN, Research Aquatic Biologist;
L.R. GOODMAN, Research Biologist

Life-cycle toxicity tests with fish provide important data necessary to evaluate the environmental hazard of pesticides and other substances. Such tests may require several months to complete. Tests of shorter duration, however, can provide initial estimates of chronically acceptable concentrations.

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TABLE 2. ACUTE (96-HR) FLOW-THROUGH TOXICITY TESTS

Pesticide	Species	Measured LC50, µg/ℓ (95% C.I.)	Mean Bioconcentration Factor
DEF	*Penaeus duorarum*	13.7 (10.5–18.4)	ND[1]
	Cyprinodon variegatus	>438	220
	Lagodon rhomboides	286.2 (237–374)	343
	Leiostomus xanthurus	127.5 (101.5–165.6)	67
EPN	*Penaeus duorarum*	0.29 (0.1–1.1)	ND
	Cyprinodon variegatus	188.9 (150.0–255.2)	-
	Lagodon rhomboides	183 (14.7–23.5)	774
	Leiostomus xanthurus	25.6 (19.2–34.2)	205
Methyl Parathion	*Penaeus duorarum*	1.15 (0.91–1.40)	ND
Phorate	*Mysidopsis babia*	0.31 (0.22–0.43)	-
	Penaeus duorarum	0.11 (0.08–0.16)	ND
	Cyprinodon variegatus	1.28 (0.97–1.74)	ND
	Leiostomus xanthurus	3.91 (3.11–5.62)	127
Trithion	*Penaeus duorarum*	0.47 (0.37–0.66)	Lost sample
	Leiostomus xanthurus	>178	260

[1]ND = nondetectable in tissues

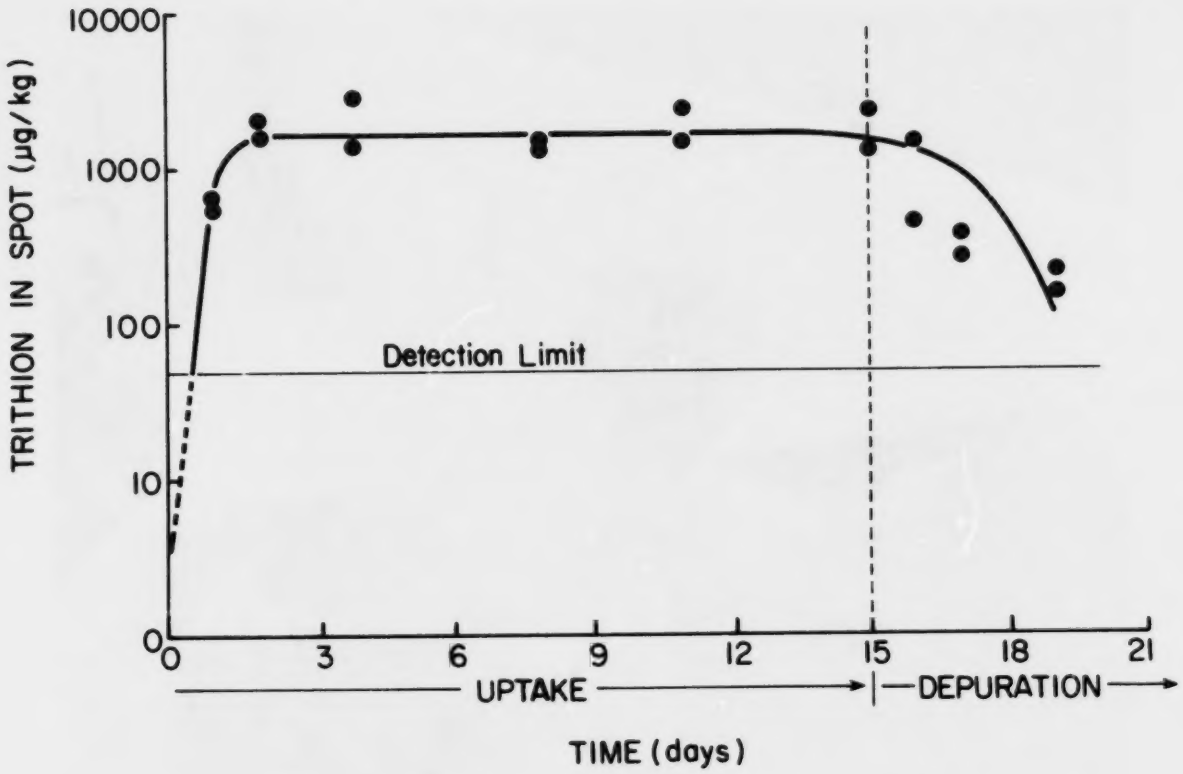

Figure 8. Bioconcentration of the insecticide Tritbion by the estuarine fisb, spot (Leiostomus xanthurus) *in a 15-day uptake, four-day depuration study. Trithion exposure concentrations were 50 µg/ℓ and 5.0 µg/ℓ.*

The search for "rapid tests" using saltwater fishes is complicated. Few life-cycle tests have been completed and only one saltwater species, the sheepshead minnow *(Cyprinodon variegatus),* has been successfully tested. In 1978, life-cycle tests were continued with the sheepshead minnow to expand the data base for reference to "short-cut" methods such as embryo/juvenile, behavioral, or physiological tests. "Short-cut" testing utilized chemicals previously tested in life-cycle tests. Such tests include: embryo/juvenile tests lasting 28 days, measurements of stamina, and measurement of acetylcholinesterase in fishes exposed to organophosphate pesticides.

Life-cycle Tests

Sheepshead minnows were used in 1978 in partial life-cycle or entire life-cycle toxicity tests with toxaphene, EPN, and Guthion. Although data from these tests have not been analyzed statistically, visual examination of the data suggests the maximum acceptable toxicant concentrations (MATC's) shown in Table 3.

In addition to measuring the effects of Guthion, EPN, and diazinon on survival, growth, and reproduction in life-cycle toxicity tests, the enzyme acetycholinesterase (AChE) was monitored to determine if it can be used as an indicator of chronic effects of organophosphate or carbamate pesticides. The diazinon test was completed in 1978, and the Guthion test is in progress. Data for the EPN test have not been statistically analyzed.

Egg production (basis of the maximum acceptable toxicant concentration [MATC] in the diazinon experiment) may have been impaired in the EPN experiment, and apparently was impaired in the Guthion experiment.

These three experiments indicate that the average number of eggs per female per day may be reduced concurrently with AChE activity of about 20% of control activity. However, egg production in the diazinon experiment was impaired with AChE activity of 78% of control activity. MATC's and AChE activity will be compared with data from EPN and Guthion experiments.

Figure 9. Biological Aide K.J. Butler exposes sheepshead minnows to industrial effluents in acute static toxicity test.

Embryo/Juvenile vs Life-cycle Tests

Embryo/juvenile toxicity tests with the sheepshead minnow may provide as reasonable an initial estimate of a maximum acceptable toxicant concentration (MATC) as that obtained from life-cycle toxicity tests (Table 3). The duration of embryo/juvenile tests is usually 4 weeks, as compared to the 12 to 24 weeks required for a partial or complete life-cycle test. Tentative results of embryo/juvenile tests predicted MATC's in life-cycle tests within a factor of 5 for 75% of 9 chemicals tested. Three chemicals for which embryo/juvenile tests poorly predicted the MATC's caused adverse effects on reproduction or growth of progeny in life-cycle tests.

Although tests conducted at other laboratories have demonstrated an excellent correlation between "no-effect concentrations" in embryo/larval tests and MATC's in life-cycle tests with freshwater fishes, ERL,GB researchers believe that additional testing on marine fishes is necessary before results from embryo/juvenile or other "rapid tests" can be substituted for results from life-cycle tests. Future tests have been recommended in these areas: (1) additional life-cycle exposures using the sheepshead minnow; (2) development of embryo/juvenile (larval) and life-cycle methods for additional marine fishes; (3) examination of additional "rapid tests" as predictors of life-cycle MATC's.

TABLE 3. ESTIMATED MATC's FOR THE SHEEPSHEAD MINNOW *(CYPRINODON VARIEGATUS)*

Chemical	Geometric Mean MATC, µg/ℓ Embryo/Juvenile Tests	Life-Cycle Tests	Quotient	Most Sensitive Life Stage
Endrin	0.19	0.19	1	All
Carbofuran	18	18*	1	Fry Survival
Malathion	6	6*	1	Fry Survival
Methoxylchlor	1.7	1.7*	1	Hatching & Adult Survival
Pentachlorophenol	275	64	4.3	Parental Survival
Trifluralin	13	2.5	5.2	Eggs Spawned
Chlordane	7.1	0.6	142	Eggs Spawned
Kepone	>0.8	0.087	>9.2	Growth
Diazinon	>6.5	<0.47*	>13.8	Eggs Spawned
Toxaphene**	0.7	0.7	1	Fry Survival
Guthion**	0.6	0.3*	2	Eggs Spawned
EPN**	>10	5.7	>1.8	Survival, growth, & Eggs Spawned

*Partial life-cycle test
**Data are based on visual inspection and not statistical analyses; therefore, conclusions must be considered provisional.

Community Bioassays

M.E. TAGATZ, Research Aquatic Biologist;
J.M. IVEY, Biological Technician

A community bioassay is designed to determine the effects of a toxicant on many different types of settling benthic organisms. In 1978, ERL,GB tests sought to assess the effects of toxicants in flowing seawater on planktonic larvae that are allowed to colonize in sand-filled aquaria.

Aquaria are arranged in groups of eight (Fig. 10); all but one group are continuously exposed to different concentrations of a toxicant. After 7 or more weeks, the number and species of animals (macroinvertebrates) that developed in exposed and non-exposed aquaria are compared statistically.

Sevin®, a widely used carbamate insecticide, altered development of estuarine communities in ERL,GB tests. The harvest after 10 weeks exposure yielded 7,844 animals, representing 29 species of 7 phyla. Average number of species per aquarium was significantly less ($\alpha = 0.05$) in aquaria containing 11.1 or 103 µg/ℓ than in those containing 1.1 µg/ℓ or in control aquaria.

The amphipod *(Corophium acherusicum)* was particularly affected; significantly fewer were found in all concentrations than in the control.

A marked increase in the abundance of the annelid *(Polydora ligni)* in aquaria containing 103 µg/ℓ corresponded to a marked decrease in the number of other annelids and to a significant absence of Nemertea.

The abundant clam *(Ensis minor)* grew significantly less in length at the higher concentrations of Sevin.

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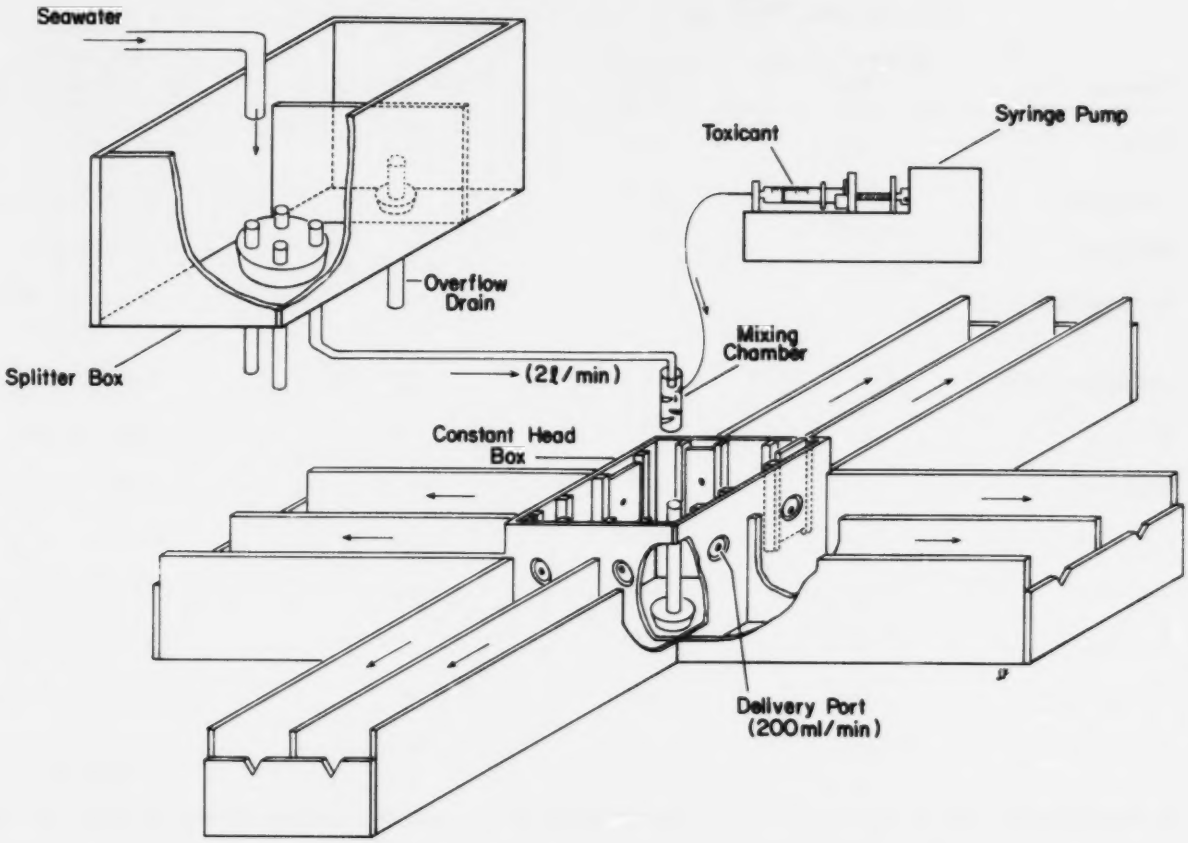

Figure 10. One of four identical apparatuses (associated with a common splitter box and consisting of eight aquaria) used to test effects of toxicants on development of macrobenthic communities.

Average lengths in mm were 8.5 (control), 8.0 (1.1 µg/ℓ), 6.7 (11.1 µg/ℓ), and 6.4 (103 µg/ℓ).

Food Chain Bioassay

L.H. BAHNER, Aquatic Research Biologist

Food-chain experiments conducted at ERL,GB attempted to determine and measure variables that could affect pesticide concentrations in predatory fish.

Experiments focused on three variables that affect pesticide transfer: (1) pesticide concentration; (2) predator feeding rates; (3) types of food consumed as a regular diet.

In the first experiment, 23,400 amphipods were contaminated with replicate concentrations of 14-C Kepone and fed to 75 juvenile spot *(Leiostomus xanthurus)* that were maintained in both Kepone-free and Kepone-contaminated water.

In the second experiment, 36 spot were fed 720, 2,160, or 3,600 amphipods that contained the same constant residues of Kepone fed to the predatory fish.

In the third experiment, mysids, amphipods, and sheepshead minnows containing similar concentrations of Kepone were fed to spot. All experiments lasted for 21 days.

Results of the first study indicate that the pathways of uptake of Kepone from water and food by spot are independent and additive. A pesticide uptake/depuration model developed at ERL,GB determined that Kepone uptake by spot from food was not affected by previous or simultaneous exposure to Kepone in water. Bioaccumulation factors for spot consuming 14-C Kepone-dosed amphipods ranged from 0.42 to 0.47.

In the second test, the equilibrium concentration ratio increased from 0.5 to 0.9 in spot fed varying amounts of food containing the same concentrations of Kepone, as the amount of food consumed increased from 1x to 5x. In the third test, results indicated that variation in the type of food consumed did not appear to be as important a factor in the equilibrium concentration as the quantity of food consumed.

Thus far, ERL,GB food chain experiments suggest that: (1) field tests can be closely simulated in the laboratory if all main variables are tested (water, sediment, food); (2) fate of pesticides in predatory animals can be predicted mathematically if the rates of pesticide transfer from one trophic level to another can be derived from 3 or 4 laboratory experiments.

Predictive Models

Stochastic Uptake/Depuration Model

L.H. BAHNER, Aquatic Research Biologist;
J.L. OGLESBY, Statistician

The bioconcentration of pesticides by estuarine animals from water and their bioaccumulation in food sources are useful indicators of pesticide movement in the natural environment. Laboratory and field studies have demonstrated that food is a prime source of pesticide contamination in certain estuarine species.

Investigators at ERL,GB in 1978 examined the impact of pesticide transfer to estuarine animals via food, water, or bottom sediment through: (1) analyses of laboratory studies and field surveys; (2) models designed to predict movements of pesticides in ecosystems.

Flow-through laboratory experiments with oysters, worms, shrimp, crabs, and fish indicated that the food-chain transfer of the pesticide Kepone was important in predicting Kepone residues in estuarine organisms. Therefore, a generalized mathematical equation was developed to describe the uptake of Kepone by such organisms. The model describes biological data as a single equation, thus allowing variations (due to many physical, chemical, biological, and random-error factors) to be analyzed simultaneously.

Kepone uptake from water, food, and sediments by estuarine invertebrates and fishes (Fig. 11) were analyzed by the nonlinear equation,
Y = P1/(1+P2**(TIME-P3)), which requires estimation of three parameters (P1, P2, and P3). The maximum predicted residue concentration (µg/g, ppb) in the exposed organism is equal to EXP(P1); the bioconcentration factor is, therefore, EXP(P1) exposure concentration. The benefits of using such a model are: (1) more realistic Bioconcentration Factors (BCF's) can be calculated from the data; and (2) differences due to exposure method or media, concentration, or time can be statistically analyzed.

(a) Plot of hypothetical uptake curves.
Model equations:

Y (i) + P1(i)/(1+P2(i)**(TIME-P3(i)))
= 7.5/(1+.8**(TIME-3))
Y (j) = P1(j)/(1+P2(j)**(TIME-P3(j)))
= 5.0/(1+.9**(TIME-1.7))

(b) Uptake of Kepone from flowing water by:

Oysters (exposed to 0.39 µg/ℓ):
Y = 7.88/(1+0.59**(TIME-1.81))

Lugworms (exposed to 0.29 µg/ℓ):
Y = 6.72/(1+0.63**(TIME-3.98))

Lugworms (exposed to 0.039 µg/ℓ):
Y = 5.01/(1+0.90**(TIME-0.45))

(c) Uptake of Kepone from food by:

Spot (exposed to 2.0 µg/g):
Y = 6.81/(1+0.79**(TIME- (–0.47)))

Blue crabs (exposed to 0.25 µg/g):
Y = 5.97/(1+0.95**(TIME-0.0))

(d) Uptake of Kepone from sediments by:

Fiddler crabs (exposed to 0.25 µg/g):
Y = 5.5/(1+0.27**(TIME-10.6))

Lugworms (exposed to 0.25 µg/g):
Y = 5.5/(1+0.73**(TIME-(–0.46)))

Blue crabs (exposed to 0.25 µg/g):
Too few data; analysis not accomplished.

Algal Stimulation and Inhibition Statistical Model

L.H. BAHNER, Aquatic Research Biologist;
G.E. WALSH, Research Ecologist

Growth of estuarine algae grown in flask cultures under controlled conditions indicates that additions of pollutant chemicals to algal growth media can cause increased growth (stimulation), decreased growth (inhibition or toxicity), or "no effect" when compared to control treatments.

Historically, straightline interpolation or probit analysis have been applied to such data for estimation of the EC50 (concentration of toxicant that inhibited growth to 50% of the untreated control); in most studies, stimulation might be noted, but not treated statistically. Since increases of minor nutrients can cause quite dramatic eutrophication, it would be beneficial to analyze stimulation data so that projections of environmental hazard can be implied.

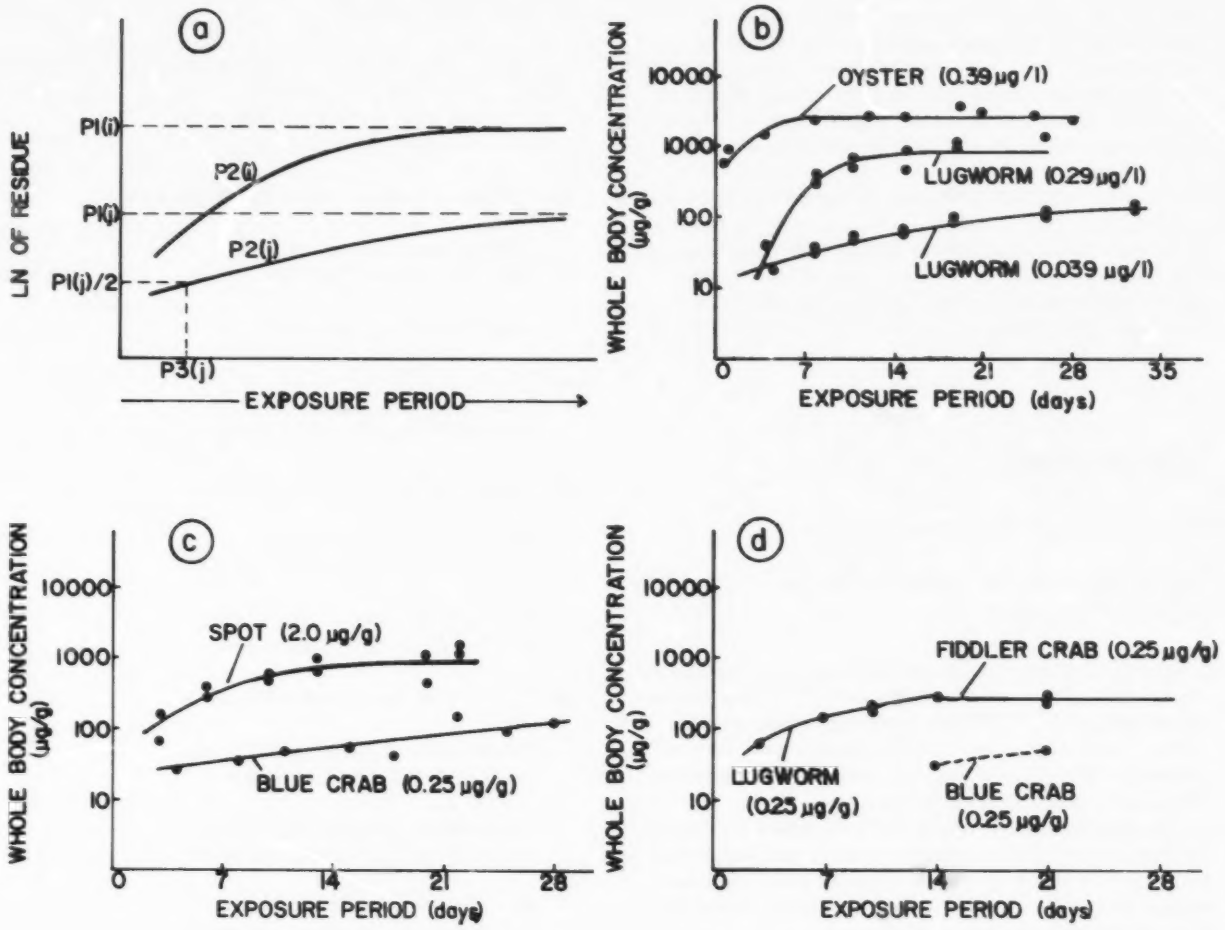

Figure 11. Uptake of Kepone from water, food, and sediment by marine organisms.

The following statistical model was designed to analyze algal stimulation and/or inhibition data:

Y = Growth Index = P6/(1+P7**(CONC-P8)) - P6/(1+P9**(CONC-P10)).

Growth Index is the dependent variable expressed as culture density, % of control, or other measure of growth;
parameter P6 is the maximum predicted growth (units are those of Y);
parameter P7 is the stimulation function slope;
CONC is the toxicant concentration of the test cultures;
parameter P8 is the concentration of toxicant that is predicted to cause growth equal to 0.5 * P6;
parameter P9 is the inhibition function slope; and
parameter P10 is the concentration of toxicant that is predicted to cause growth inhibition equal to 0.5 * P6.

Application of this model to algal bioassay data from selected industrial effluent tests is graphically illustrated to show the versatility of this model (Fig. 12) for describing stimulation or inhibition compared to control growth.

The model for algal growth was applied to data from 14 assays of textile plant effluents. Application of the model allowed concentrations of effluents that caused 20% stimulation or 50% inhibition of growth to be calculated.

Thirteen of fourteen data sets were easily modeled. Analysis could not be completed for one data set.

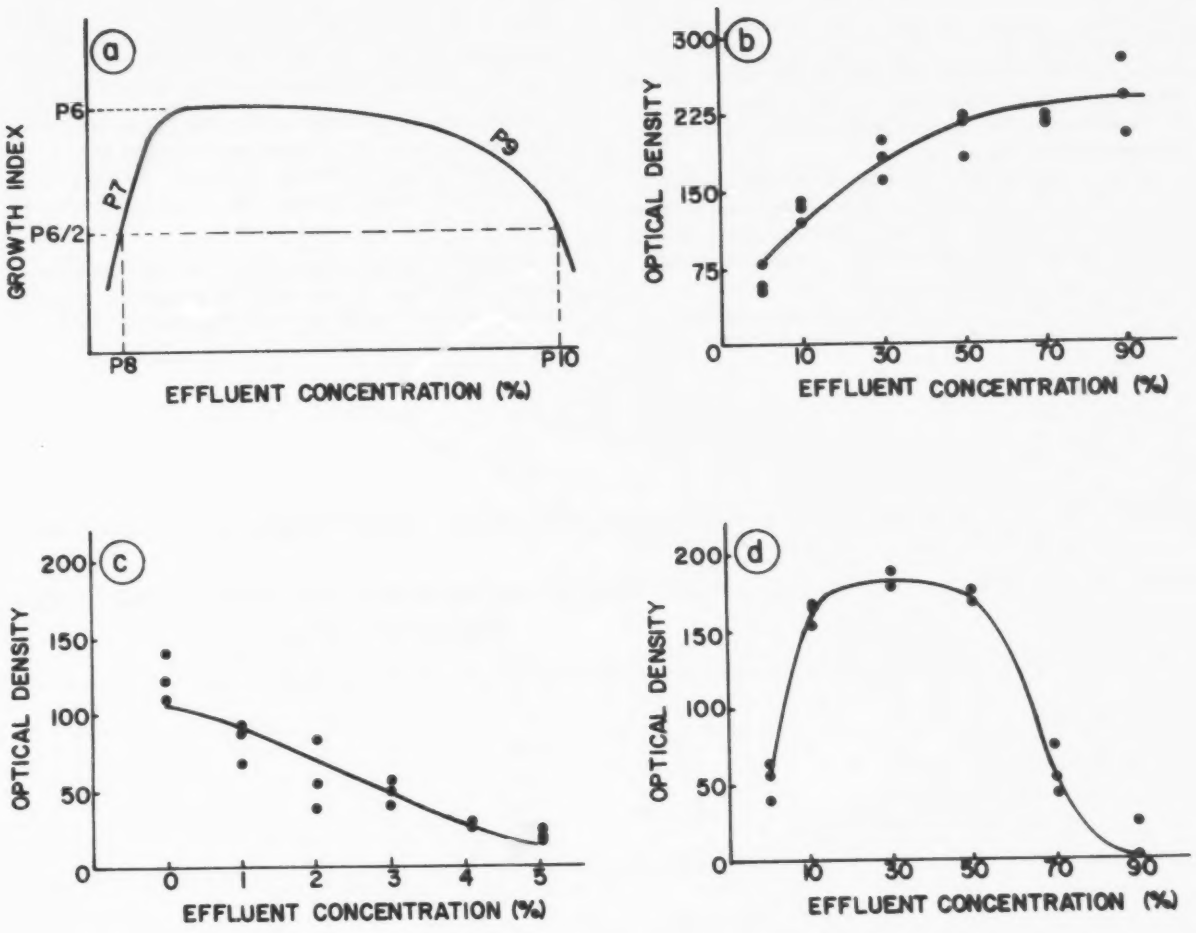

Figure 12. Effects of textile industry wastes on growth of Skeletonema costatum.

(a) Plot of hypothetical algal stimulation and inhibition of growth curve. Model equation:

Y = P6/(1+P7**(CONC-P8)) - P6/(1+P9**(CONC-P10)).

(b) Growth stimulation.

Y = 238./(1+0.94**(CONC-11.42)).

(c) Inhibition of growth.

Y = 125.-125./(1+0.47**(CONC-2.33)).

(d) Stimulation and inhibition of growth by a single waste.

Y = 182./(1+0.78**(CONC-3.49)) - 182./(1+0.84**(CONC-65.77)).

Effects of Toxicants on Selected Estuarine Flora and Fauna

GERALD E. WALSH, Research Ecologist

Industrial Waste

The Industrial Waste Program at ERL,GB is designed to identify toxic outfalls from energy-producing and other industries and to characterize the toxic wastes biologically and chemically. Biologists and chemists in 1978 devised a scheme for integrated biological and chemical tests that identify toxic fractions of liquid wastes.

Thirty outfalls from sewage treatment plants and industries such as textiles, pulp and paper, chemicals, and steel were investigated in 96-hr tests using the alga *(Skeletonema costatum)* and the mysid *(Mysidopsis babia)*. Whole wastes and their chemical fractions were examined for acute toxicity to the alga and mysid and for stimulation of growth of the alga.

Of the 30 tested, only one waste (steel plant effluent) had no effect on the organisms. Some were toxic to algae and mysids at relatively low concentrations. The response of *(Skeletonema costatum)* to whole wastes from textile industry plants is shown in Fig. 12.

Growth of *Skeletonema costatum* in whole waste and various fractions of waste from a sewage treatment plant showed that toxicity of waste was due to heavy metals (Table 4). The non-heavy metal subfraction was highly stimulatory to algae, a property of the waste that would not have been found without chemical fractionation.

Some industrial wastes tested in the laboratory were also tested in field by flow-through bioassays of *Mysidopsis babia* in a mobile laboratory. The data confirm that some wastes contain both toxic and stimulatory substances (Table 5).

ERL,GB researchers found that both toxic and stimulatory effects must be considered in assessment of potential impact of an outfall on receiving water before control technology is applied.

TABLE 4. EFFECTS OF WASTE FROM A SEWAGE TREATMENT PLANT AND ITS CHEMICAL FRACTIONS ON GROWTH OF *SKELETONEMA COSTATUM**

	PERCENTAGE WASTE	
	EC50	SC20
Whole waste	15.4	NE
Organic fraction	NE	NE
Inorganic fraction	15.5	0.4
Heavy metal subfraction	16.5	NE
Non-heavy metal subfraction	NE	0.9

*The EC50 is the calculated concentration that would inhibit growth by 50%.
The SC20 is the calculated concentration that would stimulate growth by 20%.
NE = no effect.

TABLE 5. EFFECTS OF WHOLE WASTE ON *MYSIDOPSIS BAHIA* AND *SKELETONEMA COSTATUM*

	PERCENTAGE WASTE		
	MYSIDOPSIS BAHIA	*SKELETONEMA COSTATUM*	
INDUSTRY	LC50	SC20	EC50
Kraft Mill	8.7	0.7	NE
Textiles	6.3	NE	0.08
Chemicals	13.3	2.7	79.0
Chemicals	NE	4.2	NE

Pesticides

ERL,GB maintains the capability for rapid screening of the toxicity of pesticides to algae. *Skeletonema costatum*, a chain-forming diatom, is usually the test species, but several other diatoms and green and red algae are maintained for use when needed.

Five pesticides were tested under the RPAR program for toxicity with *Skeletonema costatum*. Their 96-hr EC50's and no-effect concentrations (parentheses) are: Trithion, 0.12 ppm (0.01 ppm); DEF, 0.36 ppm (0.20 ppm); EPN, 0.37 ppm (0.01 ppm); Phorate, 1.29 ppm (0.01 ppm); and methyl parathion, 5.00 ppm (1.00 ppm).

Water Quality and Eutrophication Studies in Santa Rosa Sound

G.A. MOSHIRI, Principal Investigator; EPA Grant R805366, University of West Florida, Pensacola, FL; G.E. WALSH, Project Officer

Water samples are collected biweekly from the surface, mid-depth, and bottom of five stations near the ERL,GB laboratory. The same stations are also monitored over 48-hr periods in summer, fall, winter, and spring. Parameters such as BOD, COD, and concentrations of oxygen, nitrogen, phosphorous, and chlorophyll were measured; detailed description analyses of the phytoplankton assemblages are being made on a seasonal basis.

Life-Cycle Tests (Mysid)

D.R. NIMMO†, Research Ecologist;
T.L. HAMAKER, Biological Technician;
E. MATTHEWS, Biological Laboratory Technician

In 1978, ERL,GB investigators continued to build the data base of the chronic effects of pesticides on the life-cycle of mysid shrimp (*Mysidopsis bahia*).

In response to requests from the Office of Pesticide Programs (OPP), four RPAR pesticides were tested for chronic toxicity to mysids. The results of these 28-day tests showed mysids to be highly sensitive to these pesticides (Table 6).

In each test, the number of offspring per female was used to determine the MATC (estimated maximum acceptable toxicant concentration) values. As in the past, this criterion appears to be the most sensitive indicator of chronic toxicity of pesticides to mysids. A no-effect concentration could not be determined for the organophosphate defoliant DEF.

Life-cycle tests to examine the effect of turbidity on mysids in flowing water were undertaken in 1978 in response to requests by the Florida Department of Environmental Regulation. Clean bottom sediments from East Bay, near Pensacola, FL, were tested. Preliminary tests indicated that turbidity at approximately 1 g/ℓ of sediment affected the reproductive success of mysids. Further development of this flow-through test method could be of importance in testing the effects of various complex effluents and sediments on the life-cycle of mysids.

TABLE 6. RESULTS OF TOXICITY TESTS WITH *MYSIDOPSIS BAHIA*

Compound	96-hr LC50*	MATC*	AF**
DEF	4.36	0.34	0.08
EPN	3.24	0.44–3.24	0.14–1.0
METHYL PARATHION	0.78	0.11–0.37	0.13–0.47
PHORATE	0.37	0.09–0.21	0.24–0.57

*Micrograms per liter (μg/ℓ).
**Application factor (AF) limits are derived by dividing the Maximum Acceptable Toxicant Concentration limits by the 96-hr LC50.

†Current address, Environmental Research & Technology, P.O. Box 2105, 1716 Heath Parkway, Fort Collins, CO 80522

Figure 13. Biological Aide R.D. Fink counts grass shrimp larvae to determine chronic effects of Trithion on survival and length of larval development (time from hatching to completion of metamorphosis).

Life-cycle Toxicity Tests (Decapod Crustaceans)

D.B. TYLER-SCHROEDER, Research Biologist

The grass shrimp *(Palaemonetes pugio)* was exposed to the organophosphate, Trithion, at various life stages to determine its usefulness in assessing the toxicity of pollutants.

Prior to the life-cycle experiments, a series of 96-hr acute exposures were conducted with field-collected and laboratory-reared juveniles to establish concentrations for the life-cycle exposures and to contrast sensitivity of the two groups of shrimp. The 96-hr LC50's were: 3.4 µg/ℓ for field shrimp and 2.8 µg/ℓ for laboratory-reared shrimp.

In 1978, the flow-through system for life-cycle tests was improved by the addition of a diluter that allowed fresh water or high-salinity brine to be added to the ambient, filtered seawater. The diluter allowed greater control of salinity during freshets or periods of extreme fluctuations in salinity. Salinity is viewed as a critical factor in the survival of grass shrimp during larval development.

Although tests are not complete, preliminary results indicate that long-term effect concentrations of Trithion on grass shrimp lie close to the 96-hr LC50 concentration established in the laboratory (Fig. 13). As in earlier tests, the shrimp appeared to be most sensitive to the toxicant during their reproductive cycle. Criteria for effects on reproduction included: the number of females spawning, the number of eggs laid per female, and the number of larvae hatching per female in each concentration.

Figure 14. Research Biologist R.A. Rigby removes oyster from Sediment Bioassay System to measure growth rate.

Effects of Kepone on Development of the Blue Crab and Mud Crab

C.G. BOOKHOUT and J.D. COSTLOW, Jr., Principal Investigators; EPA Grant R803838, Duke University, Durham, NC;
D.B. TYLER-SCHROEDER, Project Officer

Experiments were conducted to determine the effect of Kepone on the development of the blue crab *(Callinectes sapidus)*, from the time of hatching until the 1st crab stage. For comparison, the effects of Kepone on larval development of the mud crab *(Rhithropanopeus harrisii)* also were investigated.

Of the concentrations tested, 35, 50, 65, and 80 parts per billion (ppb) Kepone were found to be sublethal; 95, 110, and 125 ppb Kepone were acutely toxic to *R. harrisii* larvae. In contrast, 0.1, 0.5, and 0.75 were sublethal, and 1.0 ppb Kepone was acutely toxic to *C. sapidus* larvae.

The duration of zoeal development in *R. harisii* and total time from hatching to 1st crab generally were prolonged with concentration. In *C. sapidus*, no significant relationship could be detected between Kepone concentration and duration of zoeal development, but there was a significant relationship to 1st crab. The developmental stages in which the larvae are particularly sensitive differed in the two species.

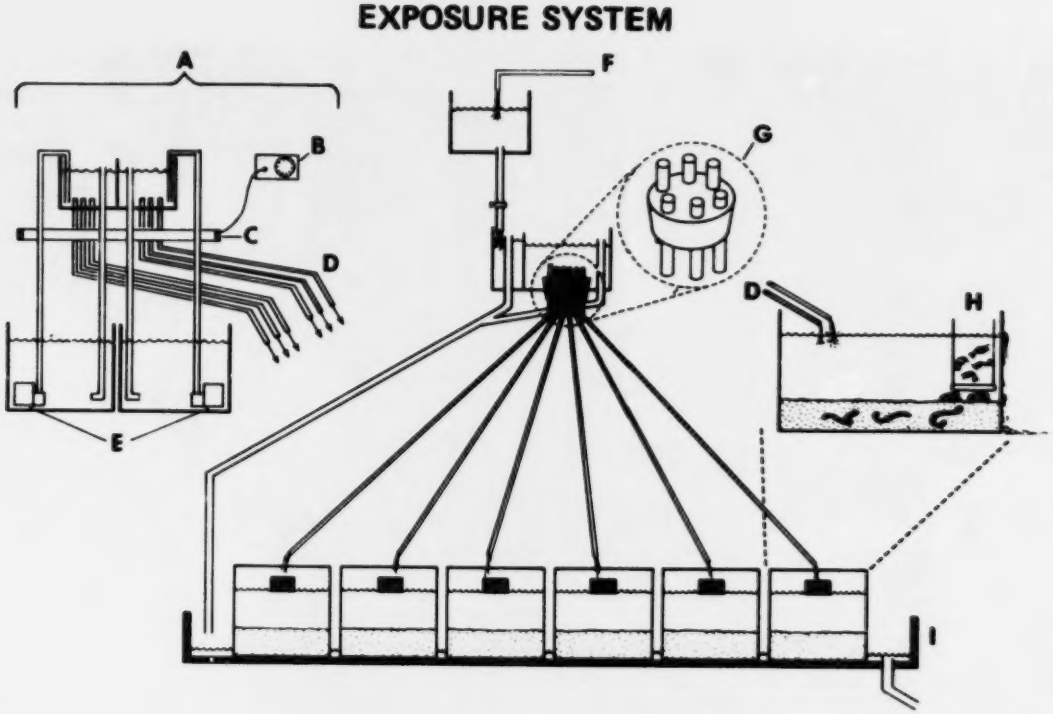

Figure 15. Sediment Bioassay System. Test sediment is introduced into 10-gallon test aquaria (H) containing bioassay organisms by means of a sediment dosing apparatus (A). Sediment is suspended by recirculating pumps (E) and is metered into the individual test aquaria from a constant head box through glass tubes (D) fitted with a length of silicon hose that is threaded through two aluminum bars (C) normally crimped shut by spring tension. The aluminum bars are attached to timer operated solenoids (B). When energized, the solenoids retract allowing the sediment water mixture to flow to the appropriate aquaria. Ambient unfiltered seawater enters the bioassay system from a head box (F) and flows to a splitter box (G) where flow rates are adjusted prior to entering the test aquaria. Effluent water is routed through a drain (I) to a holding pond.

Toxic Sediment Bioassay System

N.I. RUBINSTEIN, Research Biologist; EPA Grant R804458, University of West Florida, Pensacola, FL;
C.N. D'ASARO, Principal Investigator;
F.G. WILKES, Project Officer

Dredged material proposed for disposal into the marine environment must be evaluated by criteria established and published by the EPA (Federal Register Vol. 42, No. 7, January 11, 1977) and by the U.S. Army Corps of Engineers (USCE) and EPA (Implementation Manual for Section 103, PL92-532, Marine Protection Research and Sanctuaries Act of 1972).

An ERL,GB research team in 1978 developed a flow-through toxicity test (bioassay) (Fig. 14) to determine biological effects of contaminated sediments on representative estuarine organisms and developing benthic communities. The objective was to provide a screening tool that would detect potential hazards of dredge spoils (contaminated sediments) prior to their disposal in the marine environment.

Ten-gallon glass aquaria containing silica sand and flowing unfiltered seawater were utilized as test habitats (Fig. 15). A number of these aquaria received different concentrations of test sediments (dredge spoils); other unperturbed aquaria served as controls. Control and experi-experimental aquaria were compared to identify acute

and sublethal effects caused by exposure to test sediments.

Test organisms were selected from the three environmental compartments affected by dredging activities: mysid shrimp *(Mysidopsis bahia)*, a water-column crustacean that often scavenges on or near bottom sediments; the oyster *(Crassostrea virginica)*, an epibenthic mollusk that filters surrounding water for its nourishment; and a deposit-feeding polychaete *(Arenicola cristata*, commonly called the lugworm) that lives directly within bottom sediments and feeds primarily on the overlying detrital layer.

Test criteria were developed to identify effects of toxic sediments on: (1) survival of mysids; (2) shell deposition and bioaccumulation of known contaminants by oysters; (3) substrate-reworking and bioaccumulation by lugworms; and (4) resilience of the benthic community in terms of numbers and variety of macrofaunal organisms that settled onto test sediments from planktonic larvae within 28 days.

To date, tests have been conducted with sediment artificially spiked with the organochlorine pesticide Kepone at concentrations of 10.0, 1.0, and 0.1 ppb, and with dredged materials from the James River and the Houston Ship Channel.

Mysids, oysters, and lugworms were significantly affected by Kepone sediments. Significant reductions were detected in colonizing polychaetes exposed to the highest Kepone concentration. The pesticide was found to bioaccumulate in oysters and lugworms.

James River sediment, although not acutely toxic to test organisms, reduced oyster growth and lugworm reworking activity. These sublethal responses reflect physiological effects directly related to the metabolism of the animals. Bioaccumulation of Kepone from James River sediment was detected in lugworms and oysters at concentrations that approximated uptake from 1.0 ppm Kepone-sorbed sediment. (Subsequent analysis indicated that James River sediment contained approximately 0.5 to 1.0 ppm Kepone.) Houston Ship Channel sediment did not significantly affect test organisms in terms of the bioassay response criteria.

At present, dredge material bioassays are not considered precise predictors of environmental impact due to the number of variables that cannot be addressed in the laboratory. However, they are quantative estimators of environmental effects.

The bioassay developed at ERL,GB provides several distinct advantages over existing dredged material tests. Standard bioassay procedures are used to compare test results with an extensive data base compiled on a wide variety of marine organisms and toxicants. The test species are culturable, thus eliminating conditioning and the cost and time required for field collection of bioassay animals. The flow-through seawater design excludes many artifacts inherent to static bioassay systems and more realistically simulates actual conditions at the disposal site. Further, the use of benthic community recruitment as a test criterion provides a long-term measure of the impact of dredged material on the marine environment.

Method for Determining PCP in Marine Biota and Seawater

L.F. FAAS, Chemist;
J.C. MOORE, Chemist

A method for measuring pentachlorophenol (PCP) in the estuarine environment was described in a paper presented by two ERL,GB chemists at the 176th National Meeting of the American Chemical Society in 1978 at Miami Beach, FL.

The method uses gas-liquid chromatography (GLC) to determine PCP residues in tissues as low as 0.01 ppm by the formation of the ethyl derivative, followed by Florisil cleanup.

Seawater concentrations as low as 0.0002 ppb can be measured by formation of the amyl derivative. Formation of the amyl derivatives of PCP gives a GLC separation not possible with methyl or ethyl derivatives (Table 7). Tests using the method indicate that PCP accumulates in fish *(Mugil cephalus)*, shrimp *(Palaemonetes pugio)*, and oysters *(Crassostrea virginica)*.

Protozoan Studies

N.R. COOLEY, Research Microbiologist

Protozoa, algae, and bacteria form the basis of aquatic food chains. Of the three, ciliated protozoa are the most numerous animals in the estuarine benthos and may be more important than bacteria as nutrient regenerators, particularly of nitrogen and phosphorus. Previous work at ERL,GB and elsewhere has demonstrated that some ciliates can bioaccumulate certain persistent pesticides, thereby aiding their translocation and possible toxic effect at higher trophic levels.

ERL,GB researchers in 1978 examined the effects of toxicants, singly and in combination, on population growth of ciliate protozoa. Toxicity of nickelous chloride to *Tetrahymena pyriformis* W (grown axenically in Tetrahymena medium in flask cultures) was investigated alone and in combination with the carbamate insecticide, carbaryl (Sevin®).

When tested alone, EC50 for reduction of the 24-hr growth rate was 13.09 mg Ni^{++}/ℓ. EC50 for reduction of population size at 96 hr was 19.74 mg Ni^{++}/ℓ. When tested in combination with carbaryl, the greatest observed reduction of 24-hr population growth rate (62.35%) occurred at 15.79 mg Ni^{++}/ℓ and 20 mg carbaryl/ℓ. All combinations of carbaryl and 15.79 mg Ni^{++}/ℓ reduced the 24-hr population growth rate more than 50%. Greatest observed reduction of population size at 96 hr was 36.23% in the combination 15.79 mg Ni^{++}/ℓ and 30 mg carbaryl/ℓ.

In another investigation, the ciliate *Uronema nigricans* (strain Pc) was grown axenically at 27° C in 20 mℓ of Soldo and Merlin's M medium in 250-mℓ Erlenmeyer

TABLE 7. RETENTION TIMES OF ETHYL AND AMYL DERIVATIVES OF SEVERAL PHENOLS AND ACIDS RELATIVE TO ALDRIN ON THREE DIFFERENT GLC COLUMNS.

Compound	2% SP2100		0.75% SP2250: 0.97% SP2401		5% QF-1	
	Ethyl	Amyl	Ethyl	Amyl	Ethyl	Amyl
2,4,6-Trichlorophenol	0.14	0.40	0.13	0.34	0.17	0.40
p-Nitrophenol	0.17	0.52	0.25	0.65	0.55	1.38
2,4,5-Trichlorophenol	0.20	0.54	0.20	0.51	0.28	0.64
Dicamba	0.27	0.72	0.32	0.85	0.51	1.19
2,3,4,6-Tetrachlorophenol	0.28	0.79	0.27	0.73	0.34	0.79
2,4-D	0.38	0.98	0.48	1.24	0.79	1.82
Silvex	0.57	1.41	0.63	1.58	0.96	2.06
Pentachlorophenol	0.55	1.54	0.56	1.52	0.64	1.47
2,4,5-T	0.62	1.64	0.80	2.11	1.20	2.70
Tetrachlorohydroquinone	0.65	5.37	0.68	5.30	0.83	4.45
Aldrin	1.00	1.00	1.00	1.00	1.00	1.00

flasks and studied as a possible test organism. The tests used sodium lauryl sulfate as a possible reference chemical for future toxicity tests and as a means of determining whether response of this ciliate strain to toxicants will change during prolonged maintenance in stock cultures. The EC50 for reduction of 24-hr population growth rate was 1.78 mg/ℓ, and the EC50 for reduction of the 96-hr population size was 7.94 mg/ℓ.

Cyclic Burrowing Behavior of Pink Shrimp

C.R. CRIPE, Research Biologist; EPA Grant R804458, University of West Florida, Pensacola, FL;
C.N. D'ASARO, Principal Investigator;
F.G. WILKES, Project Officer

Pink shrimp *(Penaeus duorarum)* are among the most sensitive organisms available for aquatic toxicity testing. *P. duorarum* normally remain buried in the substrate during the day and emerge at night. Exposure to sublethal pesticide levels in ERL,GB laboratory tests have caused this aquatic species to alter its burrowing pattern by remaining continuously above the substrate. Such behavior in the natural environment would render pink shrimp more susceptible to predators.

An automated apparatus used earlier at ERL,GB to monitor the avoidance of a toxicant gradient by a test animal was adapted in 1978 to studies of abberant behavior in burrowing animals exposed to the pesticide, methyl parathion. Infrared light sources, sensors, and a microprocessor were used to monitor elapsed time of light beam interruption.

Both halves of two troughs were filled with sand and compartmentalized into four areas by plastic screens. A shrimp was placed in each area and monitored by two light beams for 6 days. A regime of 12 hr darkness/12 hr light was maintained throughout the test.

One trough was exposed to 2 ppb methyl parathion on days 3 and 4 of a 6-day preliminary test. The other trough containing four shrimp served as a control. Two of the four exposed shrimp died. The remaining two were observed above the sand during the daylight period. The activity of one surviving exposed shrimp is shown in Fig. 16.

If normal burrowing behavior is altered in an aquatic environment, the surviving shrimp would be more vulnerable to predators that feed in daylight. A reduction in shrimp population through increased predation might diminish this important commercial seafood.

Preliminary testing demonstrated that the automated apparatus developed at ERL,GB is useful in quantifying aberrant behavior in pink shrimp (Fig. 17).

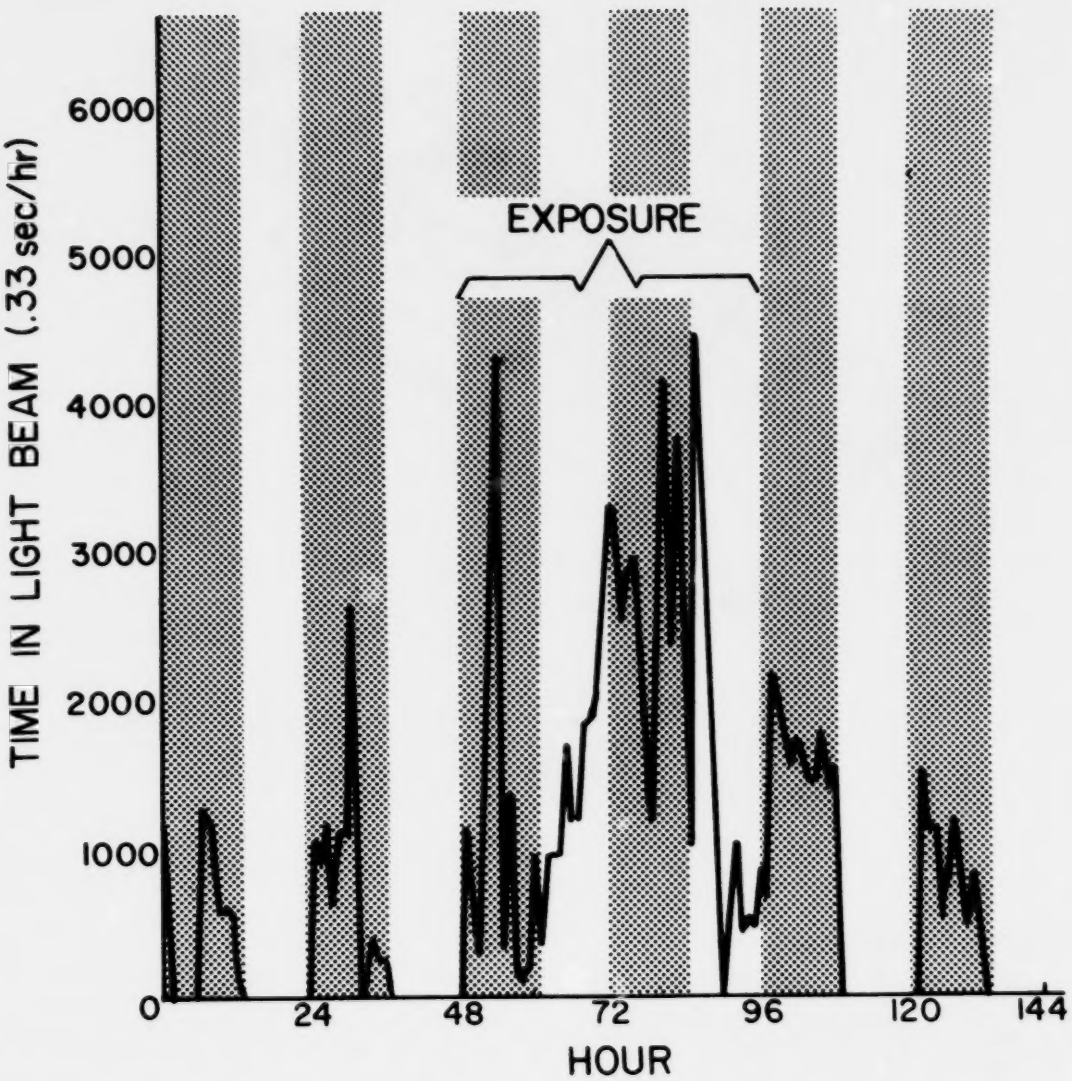

Figure 16. Activity graph indicating time and light beams (.33 sec/hr) during each hr of a 6-day test. On days 3 and 4, shrimp were exposed to an average 2.0 ppb methyl parathion.

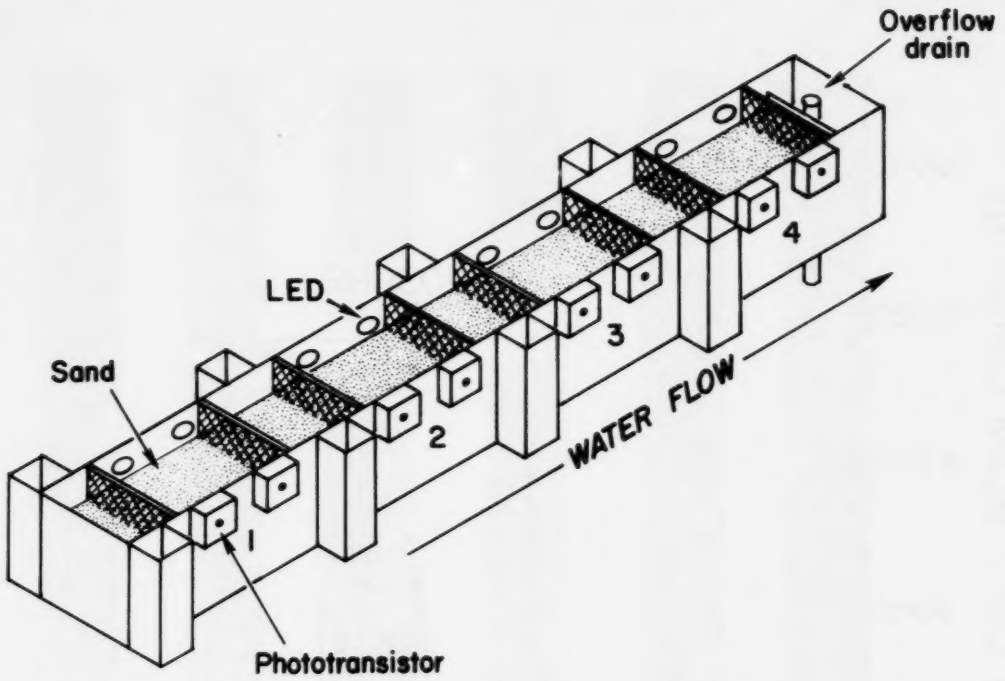

Figure 17. Diagram of one of two replicate troughs used to study pink shrimp burrowing behavior. The trough is partitioned with barriers of Plexiglas and plastic screen. The presence of each shrimp above the sand is monitored by two pairs of phototransistors and infra-red LED's.

Predator-Prey Bioassay System

C.R. CRIPE, C.E. ASHTON, Research Biologists; EPA Grant R804458, University of West Florida, Pensacola, FL; C.N. D'ASARO, Principal Investigator; F.G. WILKES, Project Officer

Earlier ERL,GB studies on effects of toxicants on an estuarine predator-prey relationship have indicated that exposure to certain sublethal pesticide concentrations increase vulnerability of the prey. Test systems were modified in 1978, focusing on the effect of the toxicant on: (1) prey only and (2) exposed and control prey in the same tank.

Three pinfish *(Lagodon rhomboides)* and equal numbers of toxicant-exposed and control grass shrimp *(Palaemonetes pugio)* were placed in two replicate tanks (208ℓ) containing removable dividers. Approximately 20 min after the dividers were removed, the surviving shrimp were counted to determine differential predation between exposed and control prey. Preliminary tests indicate that marking the prey by removal of the first right or left pleopod produces no significant effect on predation.

Significantly fewer ($\alpha \leq 0.01$) shrimp survived predation after exposure for 24 hr to 1.2 ppb methyl parathion. Exposure to 1.3 ppb Trithion for 24 or 72 hr produced no significant difference in predation.

Role of Benthic Invertebrates on Fate and Transport of Xenobiotics

C.R. CRIPE, C.E. ASHTON, Research Biologists; EPA Grant R804458, University of West Florida, Pensacola, FL; C.N. D'ASARO, Principal Investigator; F.G. WILKES, Project Officer

The fate of xenobiotics in estuarine systems is determined by the quantity added and amount of dilution, degradative processes (i.e., photolysis, hydrolysis, and metabolism), sorptive processes, biological accumulation, and transport phenomena.

Little information exists on the relative importance of benthic invertebrates on the fate of xenobiotics as compared to other processes.

Previous studies at ERL,GB indicate that benthic invertebrates can have a significant effect on pollutant mobilization into the sediment in relatively large microcosms. The role of these invertebrates is under study to determine if the same process could be demonstrated in smaller, more experimentally manageable systems. Preliminary tests used small, static estuarine water/sediment systems to which ^{14}C-methyl parathion was added as the model xenobiotic.

Replicate systems either with or without a juvenile polychaete *(Arenicola cristata)* are being compared to a formalin control to determine the relative impact of the invertebrates, microbes, or physical processes (Fig. 18).

Initial data indicate that radioactivity in the water seems to disappear more rapidly in the polychaete systems during the first week of exposure than in the other systems. These differences between the systems, however, were greatly reduced with continued incubation. Further analysis of extractable and nonextractable radioactivity may indicate whether this difference is due to degradation or transport, or both.

Figure 18. C.E. Ashton and C.R. Cripe remove samples from sediment systems.

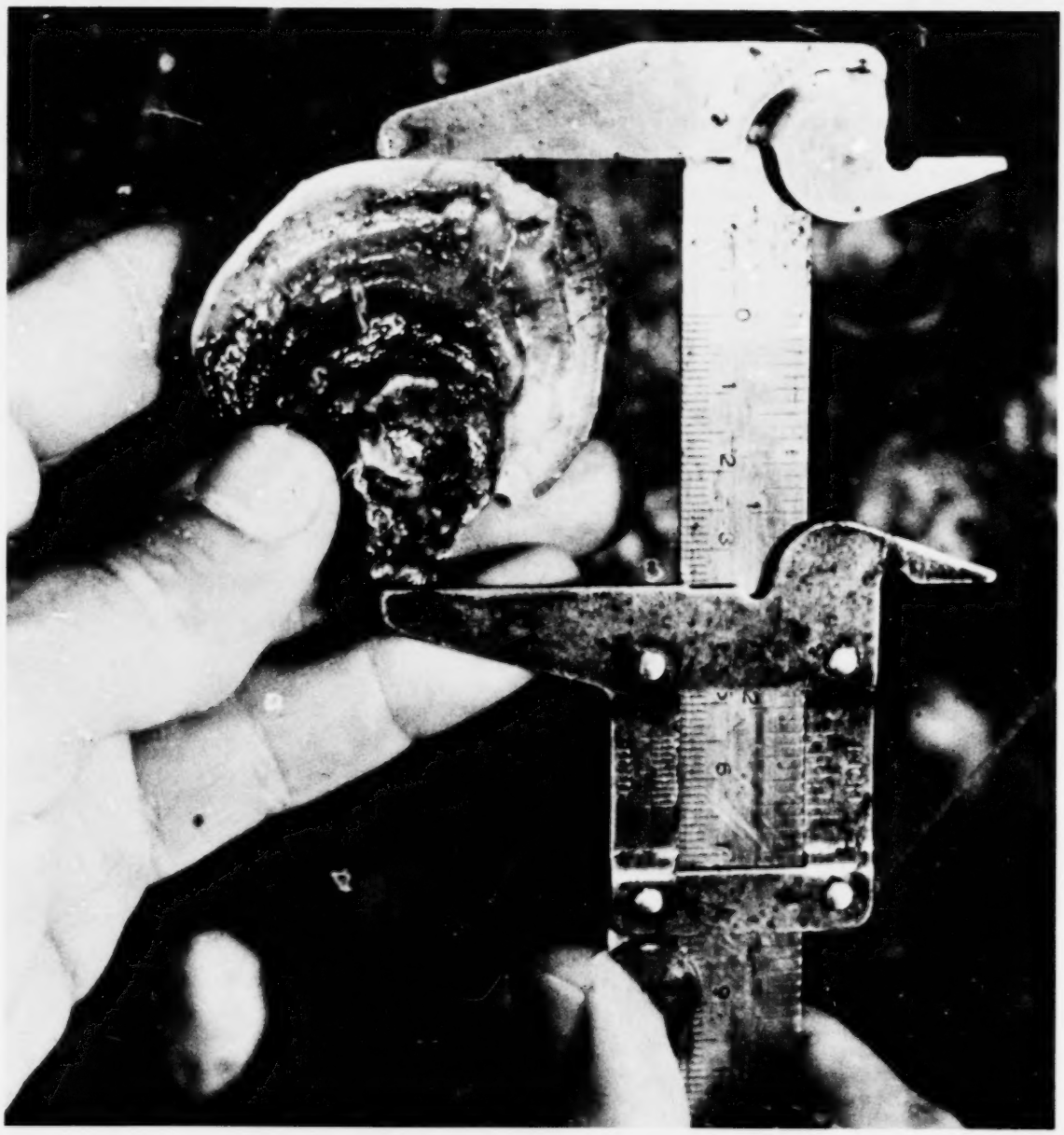

Figure 19. The American oyster is measured prior to exposure to chlorinated seawater in a study of its potential to bioaccumulate chlorination by-products.

CHLORINATION STUDIES

Large-scale use of chlorine as a disinfectant and an oxidant for drinking water and wastewaters has created concern about the toxicity of chlorination by-products to aquatic life, the persistence of chlorinated organic compounds in the environment, and their accumulation in the marine food chain.

In 1978, scientists at the Bears Bluff Field Station, Johns Island, SC, continued investigation of effects of chlorination by-products on marine/estuarine organisms, communities, and food webs.

Projects focused on effects of such compounds on the physiology and growth of oysters, interactions between low levels of chlorination and the community structure of bottom-dwelling animals, and the effects of halogenated compounds on marine phytoplankton.

Oysters

G.I. SCOTT, Biologist;
S. KLINGENSMITH, Biologist;
D.P. MIDDAUGH, Research Aquatic Biologist

American oysters *(Crassostrea virginica)* were tested to determine potential to bioaccumulate chlorination by-products (Fig. 19).

Adult oysters, 6 to 10 cm in height, were exposed in replicate exposure tanks to a nominal concentration of 1.0 mg chlorine/ℓ added as $Ca(OC1)_2$. A second group was exposed to seawater that had been chlorinated at a nominal rate of 1.0 mg chlorine/ℓ, then dechlorinated with sodium thiosulphate ($Na_2S_2O_3$).

Two other sets of replicate tanks were maintained as $Na_2S_2O_3$ and seawater controls. Tests were conducted in flow-through tanks that received seawater of ambient temperature (26° to 30°C) and salinity (22.5 to 30 parts per thousand [°/oo]) at 5 ℓ/hr/oyster for 30 days, followed by depuration for 16 days.

Mortality was 10% in the study for oysters exposed to chlorinated seawater; measured chlorine-produced oxidant (CPO) was approximately 0.20 mg/ℓ. Oysters in the chlorinated-dechlorinated tanks (measured CPO = 0.0) and controls had a 2% mortality.

Condition and gonadal indices of oysters showed changes, depending on the exposure regime (Table 8). After 16 days of exposure, the condition index of oysters maintained in the chlorinated-dechlorinated tanks was significantly lower than that of the seawater control group. Gonadal indices of oysters maintained in the chlorinated, chlorinated-dechlorinated, and $Na_2S_2O_3$ control tanks were all significantly lower than those of the seawater control group.

Chemical analyses revealed generation of chlorination by-products (predominantly bromoform, $CHBr_3$) in the chlorination exposure tanks (5.5-54.3 µg bromoform/ℓ) and in the chlorination-dechlorination tanks (4.2-38.3 µg bromoform/ℓ). A two- to three-fold biological magnification of bromoform occurred in oyster tissues, compared to the levels measured in water samples taken from the treatment tanks within a week. Oysters depurated bromoform after chlorination, and chlorination-dechlorination exposures were discontinued.

Marine Ecosystem Testing Units (METU)

P.F. SHERIDAN, Marine Ecologist;
W.P. DAVIS, Supervisory Aquatic Biologist;
R.L. YOAKUM, Biological Technician

Since 1975, Bears Bluff investigators have used the Marine Ecosystem Testing Units (METU) to observe the complex interactions between low concentrations of chlorination and community structure of benthic taxa. METU consists of 96 outdoor tanks that receive a continuous flow of estuarine water containing eggs and larvae of marine organisms. The system serves as a habitat for developing communities (algae, amphipods, gastropods, barnacles, tunicates, polychaetes, mollusks, and other groups). Communities are exposed to continuous chlorination at nominal concentrations of 0.125, 0.250, and 0.500 mg sodium hypochlorite (NaOC1) per liter (ℓ) and are compared with non-chlorinated control communities (Fig. 20).

Two series of community tests have been conducted. In the first series (28 months), communities were allowed to develop for 30, 60, or 120 days. The flow rate of seawater entering the tanks was 40 ℓ/hr.

Although the numbers of organisms in the communities increased as development time increased (from 30 to 120 days), the community composition, in terms of distribution of organisms among the various taxa, was similar under the influence of a given chlorination concentration. Intermediate concentrations of chlorination (0.125 and 0.250 mg NaOC1/ℓ) generally produced larger communities than those found in the control tanks or at the highest nominal concentration of NaOC1 (0.500 mg/ℓ) in which community densities were similar to controls. Amphipods were the most abundant organisms: *Gammarus mucronatus* and *Corophium acherusicum* predominated.

All chlorination concentrations stimulated amphipods, decapods, and bivalves. Variable responses were observed in gastropods, polychaetes, and isopods, whereas occurrence of insects, barnacles, and tunicates was depressed.

In a second series of experiments (now in progress), effects of increased flow rates and increased surface area are being investigated. Communities were allowed to develop for 60 days in the chlorination concentrations tested earlier. Seawater flow rates were doubled from 40 ℓ/hr to 80 ℓ/hr in half of the test tanks.

Preliminary results indicated increased occurrence of amphipods, polychaetes, shrimp, and tunicates, and decreased occurrence of other species, including bivalves

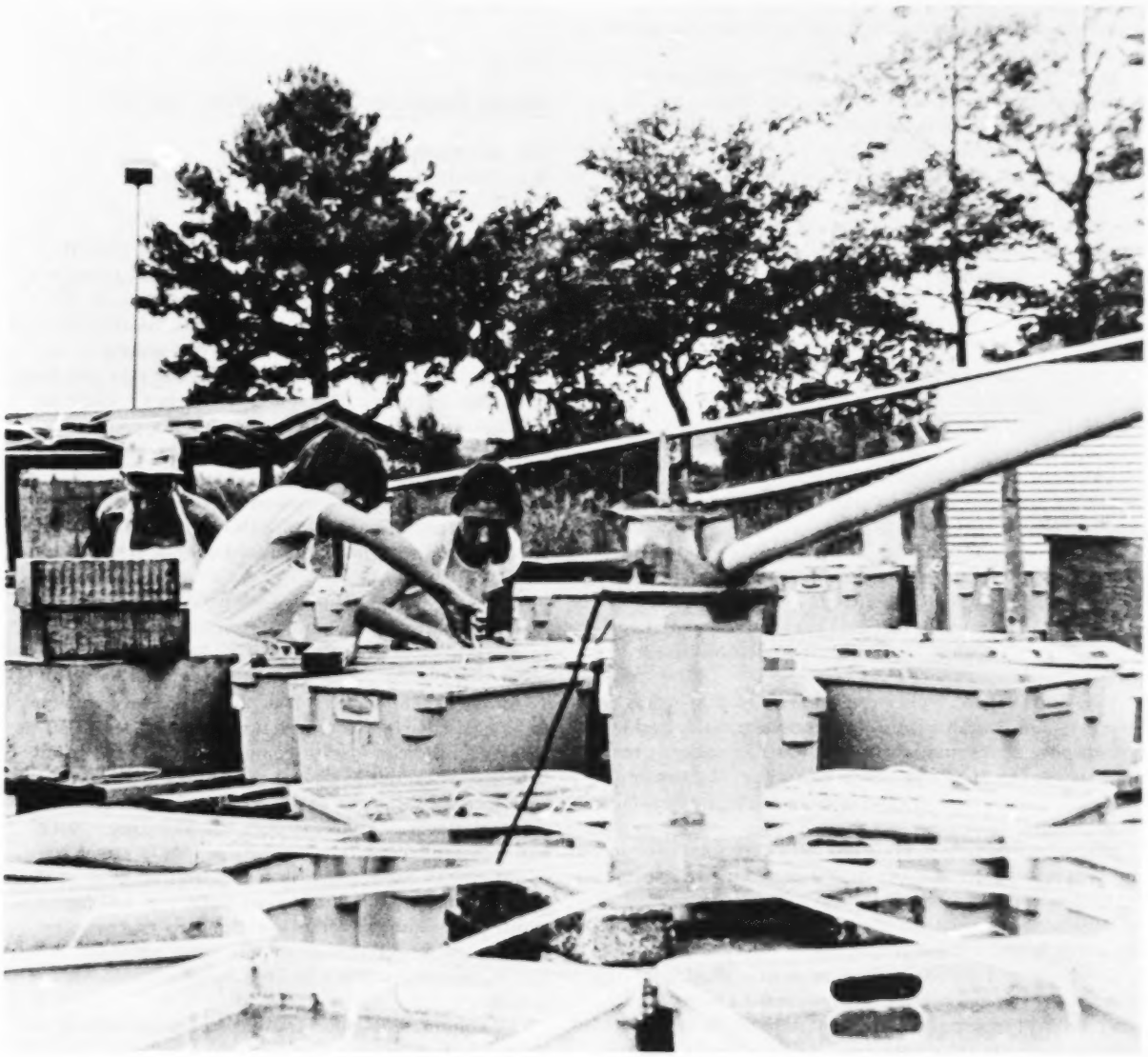

Figure 20. Biological Aides Joe Caddes, Elizabeth Bee, and Ann Hart (right) assist in harvest of aquatic species in Marine Ecosystem Testing Units (METU).

TABLE 8. CONDITION AND GONADAL INDICES FOR OYSTERS EXPOSED TO VARIOUS CHLORINATION REGIMES.

Type of Exposure*	Condition Index			Gonadal Index		
	N	$\bar{X}$	S	N	$\bar{X}$	S
A	16	3.55	0.82	16	10.1**	5.32
B	16	3.18**	1.06	16	8.8**	6.22
C	16	3.75	0.70	16	11.6**	2.85
D	15	4.10	0.67	16	16.0	3.68

*A, chlorination without dechlorination; B, chlorinated seawater dechlorinated with $Na_2S_2O_3$ before entering treatment tanks; C, $Na_2S_2O_3$ and seawater control; D, seawater control.

**Significantly different from seawater controls. $P < 0.05$.

and barnacles. Cages for sedentary organisms (i.e., mollusks) were installed in half of the test tanks to determine the effect of increased surface area. Ten times more tunicates occurred in tanks with cages than in tanks without cages; other community components showed variable responses. Effects of chlorination continued to show trends similar to those observed in the first experimental series.

Mini-METU

A.C. BADGER, Research Aquatic Biologist;
I.B. JOHNSON, Biologist

Organisms from a miniaturized experimental community configuration are being sorted and identified. Initial review of these data indicates that Mini-METU is a less sensitive toll for assessing effects of chlorination of marine communities than METU.

Fewer organisms colonized the much smaller, indoor Mini-METU tanks. Although Mini-METU tests have been halted, the system may be useful in the future for preliminary testing prior to definitive tests with the more complex METU system.

Analytical Chemistry and Productivity Studies

A.M. CRANE, Chemist;
S.J. ERICKSON, Research Aquatic Biologist

Many chlorination experiments use aqueous solutions of NaOC1 or $Ca(OC1)_2$ as a source of oxidative chlorine (HOC1 + $OC1^-$). "Residual oxidants," produced by chlorination of the various experimental media, are determined via amperometric titration.

The theoretical chemical composition of the measured residuals in chlorinated saline water is a mixture of many oxidative species of which approximately 99.8 mole % is bromine (HOBr and OBr). Chlorine comprises less than 0.2 mole % (Table 9). However, the relationship between volume of the sample (200 mℓ) and concentration of phenylarsine oxide titrant used (0.0056N) is such that 1 mℓ of titrant is equivalent to 1 ppm (by weight) of elemental chlorine ($C1^-$).

Therefore, measured residuals have been given as total residual oxidant and are expressed as mg chlorine/ℓ. In converting to mg bromine/ℓ, the reported values must be multiplied by 2.25.

Production of trihalomethanes (THM) during chlorination of North Edisto River estuarine water (salinity = 23 °/oo) with NaOCl was investigated in three experiments that addressed: (1) the capacity of filtered estuarine water to produce THM, (2) the statistical correlation between the chlorophyll *a* content of individual species of marine algae exposed to NaOCl and the concentration of THM produced, and (3) THM production rate and chlorine demand of algal cell populations.

Chlorination of 0.22 μm Millipore-filtered estuarine water (salinity = 23 °/oo) from the North Edisto River caused rapid formation of THM, comprised mainly of tribromomethane (bromoform) and chlorodibromomethane. At nominal concentrations of 10 mg chlorine/ℓ (added as NaOCl) or greater, the trihalomethane yield after 24 hr exposure remained nearly constant at 211 ± 8

TABLE 9. CHLORINE AND BROMINE COMPOUNDS EXPECTED IN SEAWATER AFTER CHLORINATION AT DIFFERENT pH's*

	MOLE PERCENTAGE OF TOTAL ADDED CHLORINE							
pH	CLO^-	HCLO	CL_2	Br_2	Br_2CL^-	HBrO	BrO^-	E.M.F.
6	$8x10^{-3}$	0.09	$9x10^{-5}$	7	10	83	0.3	+ 1.06 v.
7	$9x10^{-2}$	0.1	$1x10^{-5}$	0.7	1	95	3	+ 1.03 v.
8	0.07	0.08	$8x10^{-7}$	0.06	0.09	74	26	+ 1.00 v.
9	3	0.03	$3x10^{-8}$	$2x10^{-3}$	$3x10^{-3}$	20	77	+ 0.95 v.
10	3	0.003	$3x10^{-10}$	$2x10^{-5}$	$3x10^{-5}$	3	94	+ 0.89 v.

*From: Dove, R. A., Reaction of Small Dosages of Chlorine in Seawater, Research Report 42/70, Central Electricity Operating Board, Southeastern Region, Scientific Services Department, Southhampton, England. p. 124.

µg/ℓ. Within the chlorine concentrations tested, chlorodibromomethane production increased with increasing chlorine concentration; the bromoform concentration decreased (Table 10) and exhibited a significant linear correlation with chlorodibromomethane ($r=0.94$, $p<0.001$).

In a second series of experiments, three species of marine algae *(Isochrysis galbana), (Carteria* sp.), and *(Thalassiosira pseudonana)* were studied to determine if their chlorophyll *a* content and their individual roles in the generation of THM during chlorination of marine waters were related (Table 11). In the presence of 10^6 *Isochrysis galbana* cells/mℓ, the total THM produced by chlorination with NaOCl to a nominal 10 mg/ℓ chlorine averaged 41% greater than THM production in filtered saline waters (Table 12). The effect of *Carteria* sp. on THM production was statistically insignificant, but chlorination of seawater that contained *Thalassiosira pseudonana* decreased the total THM production by 24%. Regression analysis of data for each algal species revealed no significant correlation between THM production and chlorophyll *a* concentrations.

Determination of residual oxidants in estuarine water with known algal cell volumes and controlled chlorination concentrations and contact times revealed that THM production and the chlorine demand of the algal culture were correlated. Rapid reduction in residual oxidant level was accompanied by the production of THM at nominal chlorine concentrations of 5, 10, and 20 mg/ℓ. Fig. 21 summarizes the relationship at the 20 mg/ℓ in the presence of *I. galbana.*

Single Species Studies (Phytoplankton)

S.J. ERICKSON, Research Aquatic Biologist;
C.E. HAWKINS, Biologist

Fifteen chlorinated and brominated compounds identified as by-products of chlorination were screened against four species of phytoplankton *(Skeletonema costatum), (Isochrysis galbana), (Thalassiosira pseudonana),* and *(Glenodinium halli)* in seawater of 25 °/oo salinity at 20 ±2°C. Growth in exposed cultures was compared with that in unexposed controls after 7 days. Concentrations of the compound that inhibited cell division (50% and 25% of control) or stimulated cell division (110% of control) are shown in Table 13. The values expressed as greater than (>) were the highest concentrations tested. The four species of algae responded in a like manner and were either stimulated, inhibited, or not affected by the same compounds.

Monochloramine was the most inhibitory compound tested. Algal response to the halogenated phenols varied. Some compounds were stimulatory. At the concentrations tested, pentachlorophenol and 2,4,6-tribromoanisole were not inhibitory.

The other five compounds inhibited growth in this order of increasing inhibition: penta-bromophenol, 2,4,6-tribromoanisole, 2,4,6-trichlorophenol, pentachlorophenol, and pentabromophenol. The two most inhibitory compounds, pentachlorophenol and pentabromophenol, contained the greatest numbers of halogen atoms.

TABLE 10. AVERAGE TRIHALOMETHANE CONCENTRATIONS 24-HR AFTER CHLORINATION OF FILTERED ESTUARINE WATER WITH NaOCl.

Nominal Chlorine Concentration	Trihalomethanes Produced		
	$CHBr_3$	$CHBr_2Cl$	Total
mg/ℓ	µg/ℓ	µg/ℓ	µg/ℓ
10	180	26	206
20	188	33	221
30	177	38	215
40	146	70	216
50	136	65	201
100	119	91	210

TABLE 11. EFFECT OF THREE ALGAL SPECIES ON CONCENTRATION OF TRIHALOMETHANES, PRODUCED AFTER 1 HR IN CULTURE MEDIUM THAT CONTAINED 10 mg NaOCl/ℓ.

Algal Species	N*	Cells/mℓ	Cell Volume μ^3	Chlorophyll a µg/ℓ	THM Range** µg/ℓ
Isochrysis galbana	6	1.16×10^6	7.48×10^6	192 ± 10	+35 to +76
Carteria sp.	6	7.22×10^4	1.22×10^6	146 ± 27	−11 to +22
Thalassiosira pseudonana	6	9.21×10^5	4.77×10^6	148 ± 9	−76 to −41

*N-number of replicates.
**THM Range = concentration in medium with cells - concentration in medium without cells.

TABLE 12. TRIHALOMETHANES PRODUCED IN 1 HR BY CHLORINATION AT 10 mg NaOCl/ℓ IN CULTURE MEDIUM WITH AND WITHOUT ALGAL CELLS.

Algal Species	N	Average total trihalomethanes produced with 10^6 cells/m present µg/ℓ	Control %	Average total trihalomethanes produced in absence of algae µg/ℓ	Control %
Isochrysis galbana	6	181 ± 19	141	125 ± 25	107
Carteria sp.	6	225 ± 13	104	215 ± 25	104
Thalassiosira pseudonana	6	160 ± 16	76	165 ± 35	81

N-number of replicates.

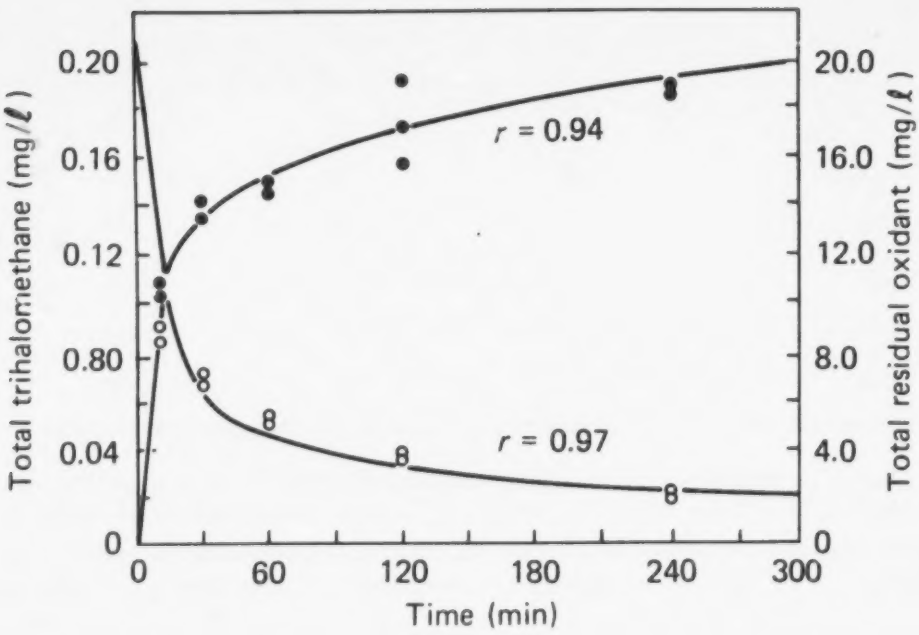

Figure 21. Total trihalomethanes and total residual oxidant as a function of time for estuarine water containing 10^6 cells/mℓ of Isochrysis galbana *exposed to a nominal concentration of 20 mg/ℓ chlorine at time zero; r is the correlation coefficient.*

All halogenated aliphatic hydrocarbons tested produced stimulatory algal growth responses; none inhibited algal cell division at the highest concentrations tested (16 or 32 mg/ℓ). The stimulatory responses observed could be the result of utilization of the compounds by the algae, or they may reflect metabolism of contaminants.

After screening tests were completed, compounds listed in Table 13 were tested in running seawater experiments to determine their effect on photosynthesis, as measured by uptake of ^{14}C, in natural assemblages of estuarine phytoplankton. Nominal exposure concentrations ranged from 0.125 to 2.0 mg/ℓ. In general, halogenated aliphatic compounds neither stimulated nor inhibited the algae, but exposure to trichloroethylene increased ^{14}C uptake to 127% of the control value. Phenol and other halogenated phenolic compounds were inhibitory (^{14}C uptake < 25% of controls). Pentabromophenol and pentachlorophenol caused greatest photosynthetic inhibition: ^{14}C uptake was 0.0 and 1.5% of controls. Haloamines formed by combining NaOCl and ammonia *in situ* caused similar inhibition.

In another study to determine the effects of continuous chlorination on entrained estuarine plankton, adenosine triphosphate (ATP) content in plankton was examined in two running seawater aquarium systems for 1 year. System A (METU) consisted of 96 37-ℓ aquaria operated outdoors and System B (Mini-METU) had 40 5.5-ℓ aquaria operated indoors. Salinities ranged from 21 to 29 °/oo, water temperature from 10° to 31°C, and the pH from 7.8 to 8.2 units during the study.

In System A, aquaria treated with a nominal concentration of 0.125 mg NaOCl/ℓ had an ATP content of 87% of the control value, 0.250 mg/mℓ, 78%; and 0.5 mg/ℓ, 67%.

TABLE 13. CONCENTRATION (mg/ℓ) OF HALOGENATED COMPOUNDS AFFECTING CELL DIVISION OF MARINE PHYTOPLANKTON*

	Stimulation by 110% of Control				Inhibition by 25% of Control				Inhibition by 50% of Control			
	Gh	Sc	Tp	Ig	Gh	Sc	Tp	Ig	Gh	Sc	Tp	Ig
Chloramine T			2	8	> 8	> 8	> 8	4	> 8	> 8	> 8	8
Sodium bromate	16	0.125	16	8	>16	>16	>16	>16	>16	>16	>16	>16
Chloroform	32	8	32	0.5	>32	>32	>32	>32	>32	>32	>32	>32
Bromoform		2	1	8	>32	>32	>32	>32	>32	>32	>32	>32
Trichloro-ethylene	16	4			>16	>16	>16	>16	>16	>16	>16	>16
Tetrachloro-ethylene	16	0.5	4		>16	>16	>16	>16	>16	>16	>16	>16
Ethylene bromide	0.5	1	0.25	2	>16	4	>16	>16	>16	4	>16	>16
2,4,6-Tribromo-anisole	0.5	0.125			4	2	2	1	4	4	4	1
p-Bromophenol		0.5	2		> 8	4	> 8	1	> 8	4	> 8	1
p-Chlorophenol	1	2	1	8	> 8	> 8	> 8	> 8	> 8	> 8	> 8	> 8
2,4,6-Tribromo-phenol					16	>16	16	16	>16	>16	16	>16
2,4,6-Trichloro-phenol					4	8	2	0.25	4	8	4	0.5
Pentachloro-phenol					0.5	1	0.5	0.25	1	2	0.5	0.25
Pentabromo-phenol					1	1	1	0.063	1	1	1	0.063
Phenol			16		16	2	>16	>16	>16	>16	>16	>16
Monochlora-mine					0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125

*Ig = *Isochrysis galbana;* Sc = *Skeletonema costatum;* Gh = *Glenodinium halli;* Tp = *Thalassiosira pseudonana*

Fishes, Crustaceans, and Mollusks

D.P. MIDDAUGH, Research Aquatic Biologist;
A.C. BADGER, Research Aquatic Biologist;
G.I. SCOTT, Biologist;
I.B. JOHNSON, Biologist

A research project initiated in the spring of 1976 to delineate factors controlling natural spawning in the Atlantic silverside *(Menidia menidia)* was completed. Field observations showed that sexually mature adults in the North Edisto River estuary, SC, began to spawn as seasonal water temperatures rose to 16°C or above in March. Spawning continued until June or July when water temperatures were 29° to 30°C (Fig. 22).

Silversides spawned only during daylight hours, and spawning runs were precisely correlated with time of high tide (Fig. 23). Also, the intensity of spawning runs periodically increased and decreased; maximum intensity occurred near the time of new and full moons.

Figure 22. Spawning Atlantic silverside photographed in the North Edisto River estuary.

Extensive examination of gonadal material from field-collected adults indicated that spermatogenesis and oogenesis also were cyclic and that the immature and intermediate egg stages apparently serve as a source of eggs which pass through a maturing and a final hydrated stage before spawning (Fig. 24). Females spawned once every 13 to 16 days, releasing approximately 500 eggs per spawning.

Data from the study are being used to model periodic variables that cue spawning in the silverside *Menidia* in laboratory culture. Laboratory spawning will provide a method to assess the effects of toxic substances on the reproductive potential of the silverside, an estuarine fish that serves as a food source for many commercially important fishes.

In another study, groups of embryonic grass shrimp *(Palaemonetes pugio)* were exposed to 0.1 and 0.3 mg cadmium/ℓ for 8 days prior to hatching. Other groups of embryos were cultured in uncontaminated seawater.

Prehatch exposure to cadmium had no additive effect on sensitivity of larvae to cadmium or salinity stress for 14 days after hatching (Fig. 25). Only one group of larvae, exposed to 0.1 mg/ℓ cadmium for 4 days before hatching and transferred in 0.1 mg cadmium in 10 °/oo salinity water after hatching, showed a significant decrease in survival (X^2, $P<0.05$) compared to control survival. No significant decreases in survival were observed for any larvae transferred to 15 and 30 °/oo salinity at a pre- and posthatch cadmium concentration of 0.1 mg/ℓ.

Pre- and posthatch exposure to 0.3 mg cadmium/ℓ caused significant decreases in survival of all larvae transferred to 10 and 15 °/oo salinity after hatching. Significant decreases in survival were observed for only two groups exposed before hatching and transferred to 30 °/oo salinity and 0.3 mg cadmium/ℓ after hatching.

Rivulus marmoratus: An Investigation of its Potential as a Cancer Research and Chemical Carcinogen Screening Organism

C.C. KOENIG, Principal Investigator; EPA Grant R805469, Grice Marine Biological Laboratory, College of Charleston, Charleston, SC;
W.P. DAVIS, Project Officer

The self-fertilizing hermaphroditic marine fish *(Rivulus marmoratus)* is being studied as a potential environmental monitor of teratogenetic and mutagenic effects of chemicals, chlorination by-products, or effluents. During 1978, over 1000 individuals have been raised, important data on their natural history has been obtained and a substantial wild stock population has been located.

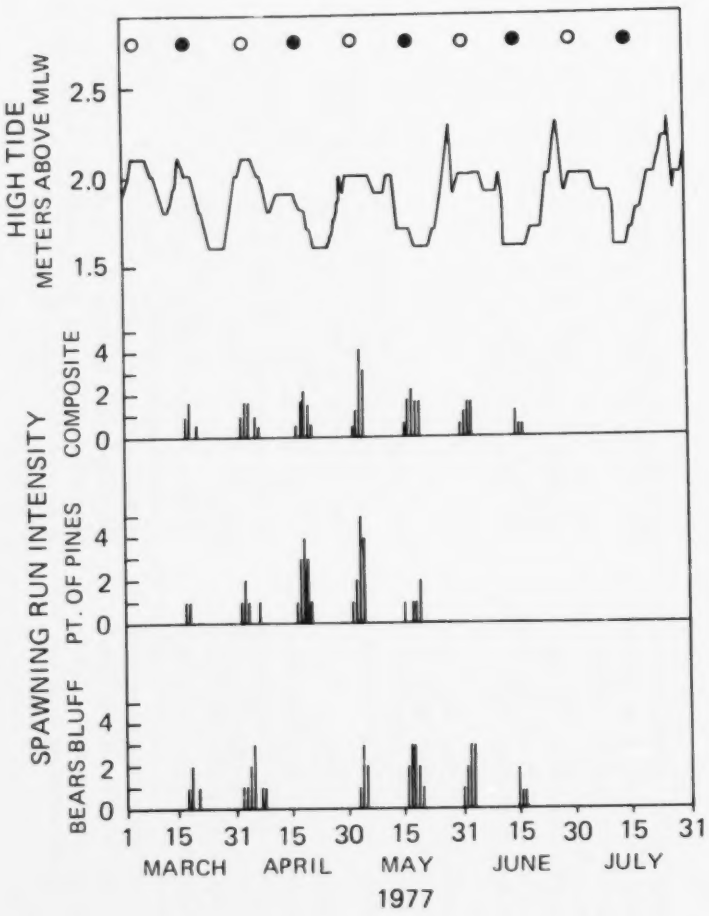

Figure 23. Daily spawning-run-intensity values at Bears Bluff and Point of Pines study sites. Composite data represent the daily mean at each site. Predicted high tide elevations in meters above mean low water (MLW) are for daytime high tides. Filled circles represent new moons and open circles represent full moons.

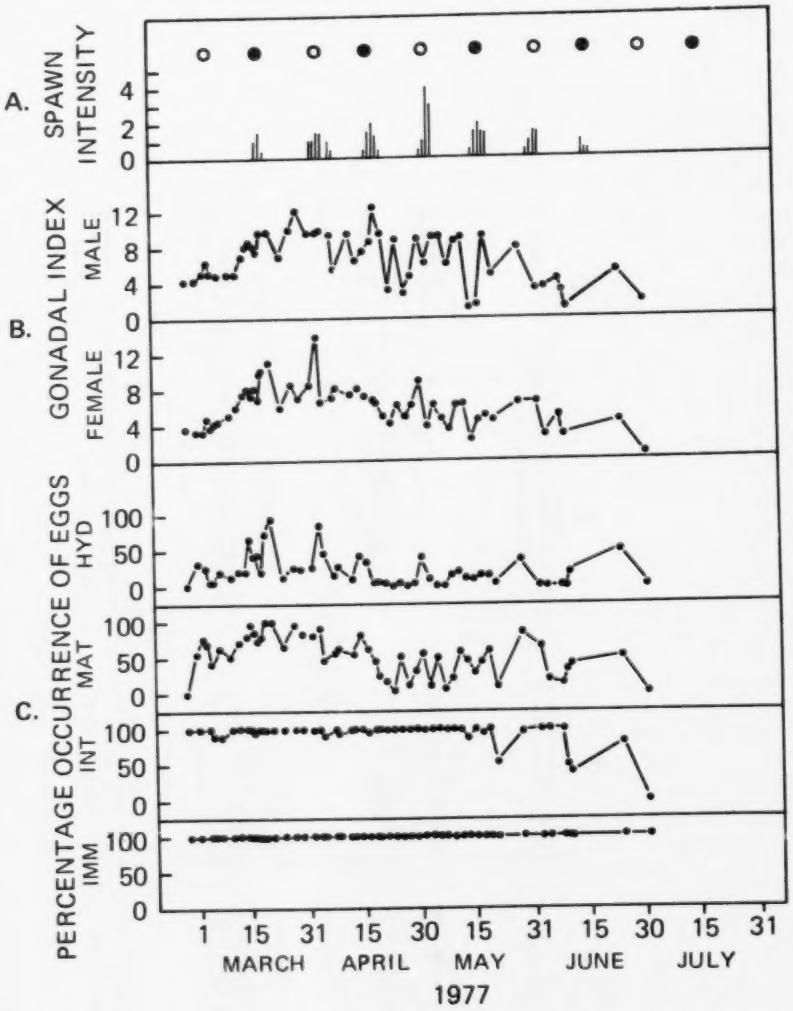

Figure 24. A. Mean daily spawning-run-intensity values for M. menidia *during 1977. Time of new moons (filled circles and full moons [open circles] are also shown; B. Mean daily gonadal index values for males and females collected during 1977; C. Percentage occurrence of immature (IMM), intermediate (INT), maturing (MAT) and hydrated (HYD) eggs in ovaries.*

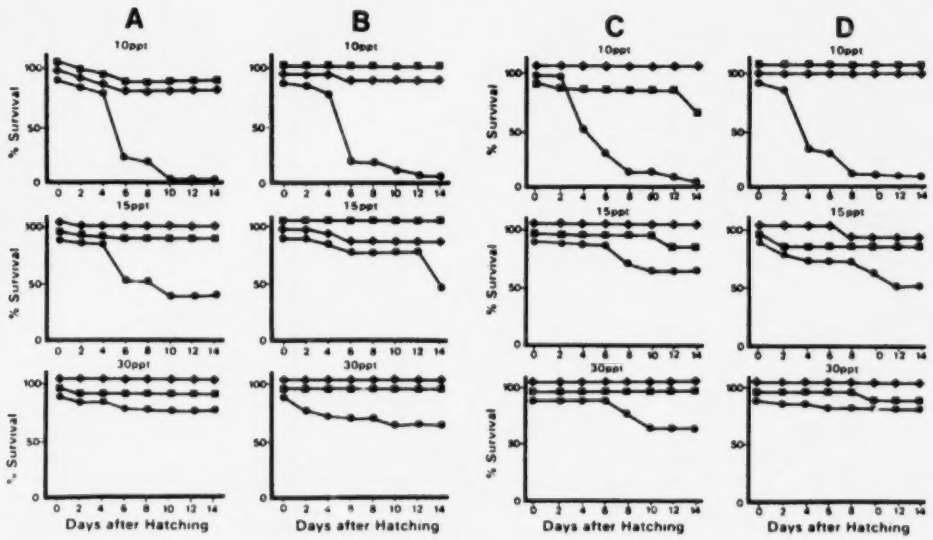

Figure 25. Survival of larval grass shrimp exposed to cadmium. A - no prebatch exposure (0 days); B - 1 day; C - 4 days; D - 8 days of prebatch exposure. Key: Control -◆ , 0.1 mg/ℓ Cd - ■ , 0.3 mg/ℓ Cd - ●

Sister chromatid exchange (SCE) is a technique successfully used in mammalian cell cultures to analyze mutagenic characteristics of chemicals or processes. Experimentation was initiated to adopt this technique to marine fishes and a method has successfully evolved. During 1979, investigations will continue to field test the method to derive a laboratory screening phase with a field verification approach.

In other activities, the culture, life-cycle, and critical life stages were examined with the bivalve mollusk *(Mulinia lateralis)*. Induced spawning was successful but larval culture under laboratory conditions has not yet been accomplished.

In addition, experiments were conducted to seek methods of laboratory maintenance of the mysid shrimp *(Neomysis americana)*. Culture of larvae to reproductive adults and production of a second filial generation were accomplished. In testing toxicity of chlorination/chlorination by-products, these organisms proved to be highly sensitive to low chlorination levels.

Spawning, egg placement, development, and maturation of the striped killifish *(Fundulus majalis)* are also under investigation. This species inhabits coastal lagoons of the southeastern Atlantic Coast and is an important food of fish-eating seabirds.

Isolation and Study of Halo-organics

J.H. CARPENTER, Principal Investigator; EPA Grant R803893, Rosenstiel School of Marine and Atmospheric Sciences, Miami, FL;
W.P. DAVIS, Project Officer

Techniques for analyzing the larger, lipid-soluble halo-organics produced by chlorination were developed and improved in 1978. Results have shown that amino acids become halo-organic by-products (Fig. 26).

Important research and regulatory questions concerning the complex process of seawater chlorination remain unanswered: (1) What is the marine environment's assimilatory capacity for exotic halo-organic by-products, and (2) What biological effects result from contact or uptake of such compounds? These answers would be applicable to decisions regarding hyperchlorination in sewage sludge stabilization, the technical management of biocide processes in electrical power generation using marine cooling water, and the disposal of municipal and industrial effluents.

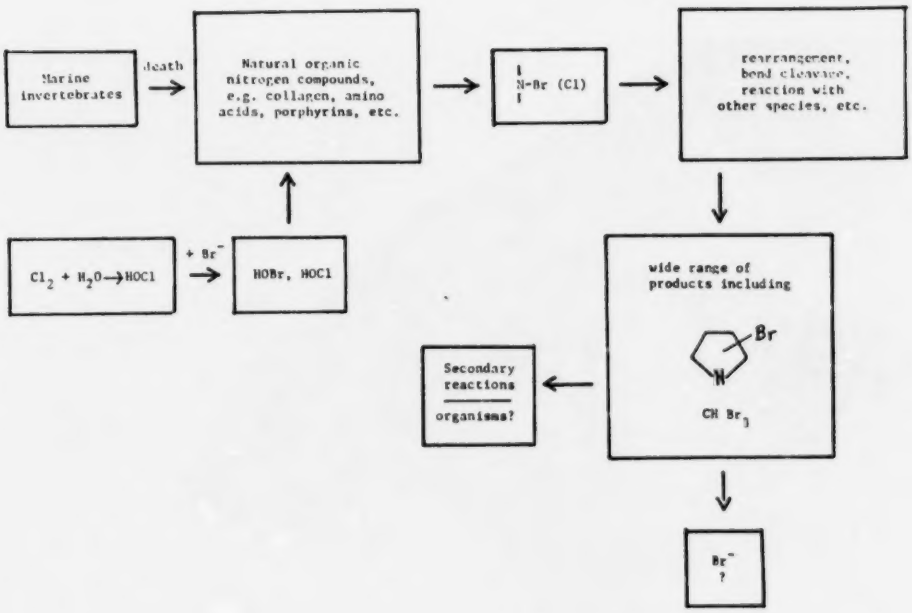

Figure 26. A possible scenario for halogenation of nitrogen containing organics in marine waters.

An Investigation of the Ecological Effects of Residual Ozone to Selected Marine Species

D.T. BURTON, Principal Investigator; EPA Grant R804683, Benedict Research Laboratory, The Academy of Natural Sciences of Philadelphia, Benedict, MD;
W.P. DAVIS, Project Officer

Effects of ozonation upon selected estuarine organisms were compared with those of simultaneous chlorination. Similar by-products of the oxidation processes and effects of oxidative compounds on invertebrates and fishes were demonstrated. Ozone has been suggested as a potential replacement for chlorination, but in terms of marine waters and production of similar THM by-products no advantage has been shown. A final report is in preparation for publication during 1979.

Food Webs, Populations, and Productivity in a Southeastern Coastal Marine Marsh

N.W. CHAMBERLAIN, Principal Investigator; EPA Grant R8044688, Grice Marine Biological Laboratory, College of Charleston, Charleston, SC;
W.P. DAVIS, Project Officer

Aquatic species in marsh ecosystems adjacent to the Bears Bluff Field Station were monitored in 1978 to provide information on their life histories, food and trophic relationships, and parasitism. Data will be used in ecosystem analysis and to validate field tests with selected pollutants or pesticides. Findings will be reported in 1979.

Ecological Response Team

W.P. DAVIS, Supervisory Aquatic Biologist;
G.I. SCOTT, Biologist;
W.P. LEMPESIS, Environmental Protection Specialist

Personnel of the Bears Bluff Field Station assisted in the National Response Team (NRT) response to the need to assess biological impacts of oil and other hazardous chemical spills in 1978 and 1979.

The NRT has coordinated emergency assistance by the Federal Government in accidental spills occurring in the U.S. and foreign countries.

The Bears Bluff scientists participated in the Federal Government's response to accidental spills in Padre Island (Texas), Brittany (France), Hackberry (Louisiana), Savannah (Georgia), Cooper River (South Carolina), Tampa (Florida), and Fajardo (Puerto Rico). A number of reports on the biological effects of these spills have been published, and a method has been defined for an integrated zonal assessment of ecological damage. The staff also assisted in designing followup research and monitoring efforts to be undertaken by appropriate regional, state, and local governments.

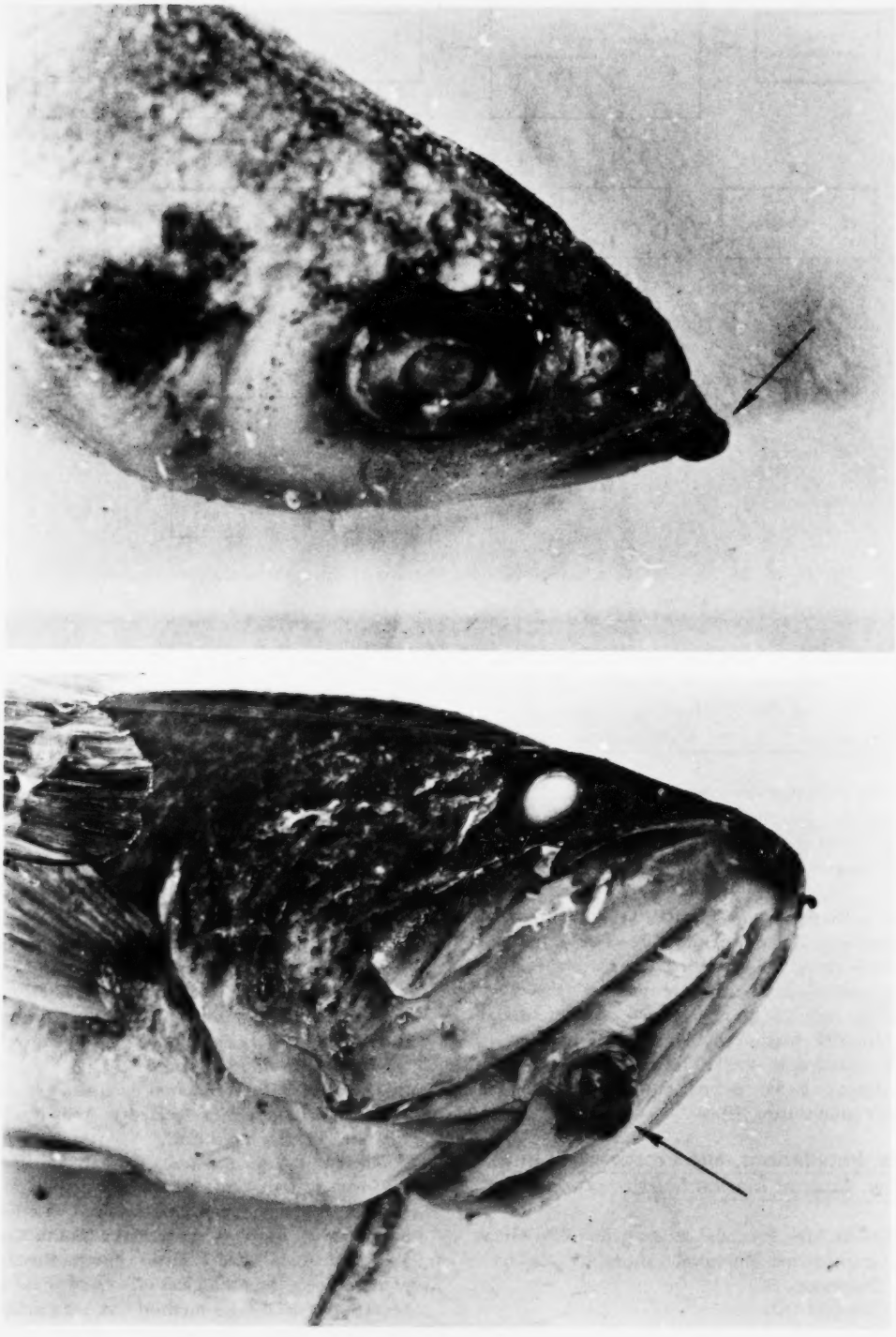

Figure 27. Arrows indicate tumors found on fish collected from Gulf Coast estuarine waters.

ENVIRONMENTAL PATHOBIOLOGY

In 1978, the Environmental Pathobiology Unit at ERL, GB initiated a new research project, "Carcinogens in the Aquatic Environment," supported by the National Cancer Institute. Three disciplines--pathobiology, biochemistry, and molecular biology--will be applied in the investigation of carcinogenic pollutants in Gulf coastal waters.

The primary mission of the research is to elucidate the first series of critical steps in the production of active carcinogens by pre-carcinogenic chemicals released in aquatic systems. Selected estuarine and marine species will be exposed to suspected carcinogens, teratogens, and mutagens.

Epizootiological Study of Tumors and Carcinogens

J.A. COUCH, Coordinator

ERL,GB scientists are evaluating the role of fish and shellfish as indicators of carcinogenic pollution in a project cosponsored by the National Cancer Institute.

Since field collections began in August 1978, several tumors and possibly neoplastic lesions have been found in fish and oysters collected in Gulf coastal waters of Florida, Alabama, and Mississippi. The types of tumors are under study to determine if specific cancer-causing pollutants can be related to tumor prevalence or cellular diseases in aquatic species (Fig. 27).

Suspected cancer-causing agents are tested in long-term exposures in a special assay system designed at ERL,GB. The system, constructed initially to test the carcinogenicy of the herbicide trifluralin, provides for the control of light, water temperature, water flow rates, and the nutritional status of test animals (Fig. 28).

Vertebral Dysplasia in Fish Exposed to Trifluralin

J.A. COUCH, Coordinator

Sheepshead minnows *(Cyprinodon variegatus)*, exposed to 5.5 to 31 µg/ℓ of the herbicide trifluralin throughout their first 28 days of life, developed a heretofore undescribed vertebral dysplasia.

This dysplasia consisted of semi-symmetrical hypertrophy of vertebrae (3 to 20 times normal), characterized by foci of osteoblast and fibroblasts actively laying down bone and bone precursors. Effects of the abnormal vertebral development were dorsal vertebral growth into the neural canal, ventral compression of renal ducts, and longitudinal fusion of vertebrae.

Fish, exposed for 51 days to 16.6 µg/ℓ trifluralin and thereafter depurated for 41 days, showed no increase in vertebral dysplasia during depuration; however, residual spinal column damage was evident. Serum calcium concentrations were elevated in adult fish exposed for 4 days to 16.6 µg/ℓ trifluralin. Fluorosis or mimicry of hypervitaminosis A are considered possible mechanisms for the osseous effect, but are not considered to be the only possible causes.

The highly predictable nature of this disorder in experimental exposures strengthens the probability that young fish may serve as experimental models for determining effects of chemicals on early vertebrate ontogeny, particularly in regard to skeletal development.

Metabolism of Polyaromatic Hydrocarbons by Mixed Function Oxidase (MFO)

P. MELIUS, Principal Investigator; EPA Grant R806213, Auburn University, Auburn, AL;
W.P. SCHOOR, Project Officer

The metabolism of polynuclear aromatic hydrocarbons (PAH) by normal and induced mullet and killifish liver homogenates and microsomal preparations was measured. The Ames test was used to identify the mutagenic metabolites of 3-MC.

Rats, mullet, and killifish were induced with either Aroclor 1254 or 3-MC; 3-MC was used as substrate. In addition, NADH-ferricyanide reductase activity was measured. Sodium dodecyl sulfate (SDS) electrophoresis was carried out in order to measure the appearance of heme-containing proteins.

In the experiment using the TA 98 and TA 100 mutants, mullet S-9 preparations, and 3-MC (25 µg) substrate, a significant increase in revertants occurred at the 100 mg Aroclor/kg dose level. For no known reason, no increase was found at the 200 mg Aroclor/kg level. Possibly the 200 mg level of Aroclor was toxic to the cells.

Under these conditions, chrysene apparently was not mutagenic. The 3-M had a very slight but erratic mutagenic effect with killifish S-9 preparations. The TA 1535 and TA 1538 organisms gave no significant mutagenesis in any of these experiments. Problems were encountered in maintaining the *Salmonella* TA 98 and TA 100 mutants without spontaneous reversion.

The NADH-ferricyanide reductase activities were measured in controls and Aroclor-treated rats, mullet, and killifish. These activities slightly increased in adult rats, as compared to neonatal rats. The 250-mg Aroclor/kg dose level in the adult rat caused a three-fold increase in enzyme activity, whereas the 200-mg Aroclor/kg dose level in the mullet caused a 30% increase in enzyme activity. These results appear to agree with those reported for earlier experiments which indicated that induced enzyme levels were significant in the rainbow-trout, but lower than in the rat.

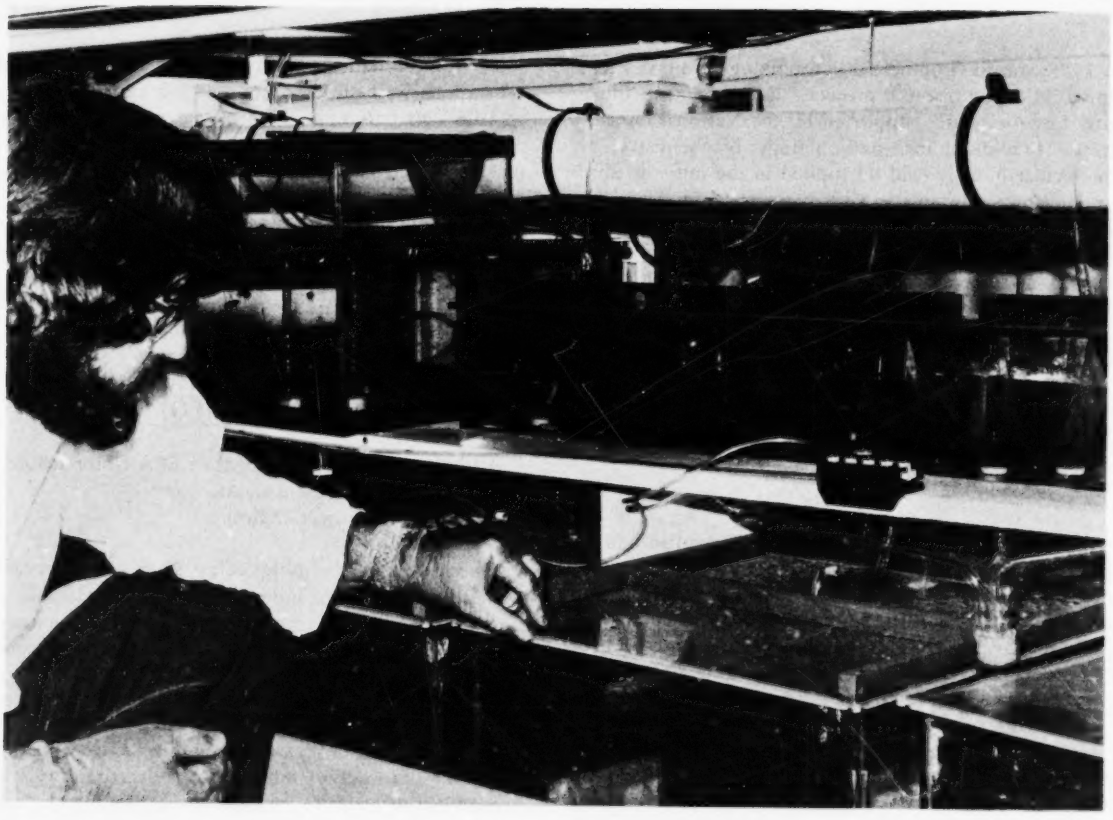

Figure 28. Visiting Investigator L.A. Courtney checks aquaria designed to test carcinogencity of the herbicide trifluralin in aquatic species.

Oxidation and Conjugation of Carcinogenic Hydrocarbons in Marine Animals

D.R. STRENGTH, Principal Investigator; EPA Grant R806368, Auburn University, Auburn, AL;
W.P. SCHOOR, Project Officer

Six enzymes were studies in the investigation of oxidation and conjugation of carcinogenic hydrocarbons: (1) UDP-glucuronosyl transferase, (2) 3-phosphoadenosine-5-phosphosulfate sulfotransferase, (3) UDP-glucose dehydrogenase, (4) glucose-1-phosphate uridyltransferase, (5) b-glucuronidase, and (6) aryl sulfatase. The criterion used for validation was a direct proportionality between reaction rate and enzyme concentration.

Systematic procedures have been developed for handling tissue samples, including the selection of appropriate buffers, proper homogenization, centrifugation for optical clarification, and use of Sephadex G-25 to remove interfering low molecular weight substances. The assay will be validated in tests with rat and marine animal tissue.

Carcinogen Assay System for Estuarine Fishes

B.J. MARTIN, Principal Investigator; EPA Grant R804527, University of Southern Mississippi, Hattiesburg, MS;
J.A. COUCH, Project Officer

A closed-circulating assay system was designed to study the effects of the carcinogenic polycyclic aromatic hydrocarbons (PAH), benzo[a]pyrene (BaP), and methylcholanthrene on sheepshead minnows and channel catfish.

Fish were maintained in the system for up to 31 weeks in weekly contaminations of PAH. Significant levels of BaP and methylcholanthrene remained in the water column for only ca. 24 hr each week. No tumors were observed in the exposed fish during the study.

The incidence and types of lesions in control and exposed fish were basically similar except in catfish that were fed PAH-contaminated food. High levels of contamination (1 mg/gm food) appeared to be toxic and lower levels of contamination (0.1 mg/gm food) produced sufficient stress to make the catfish susceptible to fatal parasite infestations.

Both species accumulated radioactively labelled PAH at concentrations much higher than their nominal concentrations in the water. Although the level of accumulation was extremely variable, the accumulation factors, in general, were: ca. 30X in gill and liver, ca. 15X in GI tract, and ca. 2X in skeletal muscle.

In an experiment in which 10 catfish were maintained in water contaminated on a weekly basis with 1.0 µg/ℓ BaP, the fish remained healthy for approximately 7 months. In the next 3 months, four of the fish became very scoliotic and lordotic and exhibited nervous disorders in their manner of movement.

Most of the affected fish also displayed abnormal melanocytic control and were much darker in color than normal fish. Radiographs (Fig. 29) illustrate the vertebral disorientations that occurred. The high incidence of this phenomenon in exposed fish that continued to feed normally and had normal growth rates suggests that a cause-effect relationship may exist between the lesion and BaP exposure.

Chemical Carcinogens in Bivalve Mollusks from Oregon Estuaries

M.C. MIX, Principal Investigator; EPA Grant R806224010, Oregon State University, Corvallis, OR;
J.A. COUCH, Project Officer

Indigenous populations of bivalve mollusks were used as monitors for detecting and quantifying environmental BaP in Oregon estuaries. Short-term and long-term studies were conducted to establish base-line levels of BaP and to identify seasonal variations in BaP concentrations in shellfish. A presumptive cellular proliferative disorder (thought possibly to be neoplastic) was also studied in mussels *(Mytilus edulis)* from Yaquina Bay.

Histological studies revealed that mussels inhabiting polluted environments had an average 6 to 8% prevalence of the cellular proliferative disorder not observed in mussels maintained in clean environments. The cellular condition followed a seasonal pattern: a low prevalence in the summer and fall was followed by an increase in early winter; prevalence peaked in January-February. The atypical, large cells that characterize the disorder in *M. edulis* possess many ultrastructural properties in common with malignant vertebrate cells.

Findings were published in March 1979 in the EPA Ecological Research Series.

Separation of Compounds with Carcinogenic Properties Found in Marine Invertebrates

C.W. CHANG, Principal Investigator; EPA Grant R806108, The University of West Florida, Pensacola, FL;
N.L. RICHARDS, Project Officer

Analytical methods will be developed for the characterization of genotoxic compounds bioaccumulated by marine organisms from the water column. A concentration and separation scheme will be designed and validated.

Investigation of Chemical Mutagen Accumulation in the Tissues of Marine Organisms

J.R. BAYLIS, Jr., Principal Investigator; EPA Grant R806339, The University of West Florida, Pensacola, FL;
N.L. RICHARDS, Project Officer

Activation and detection methods were developed and validated with diverse reference mutagens/carcinogens. These techniques will be used to screen fractions of oyster tissue extracts taken from clean and polluted waters.

Enzymatic Screening Tests for Mutagens

J.J. SCHMIDT-COLLERUS, Principal Investigator; EPA Grant R805671, University of Denver, CO;
N.L. RICHARDS, Project Officer

An enzymatic screen developed for chemical carcinogens was based on the selective *in vitro* stimulation of microsomal biphenyl-2-hydroxylase by known chemical carcinogens.

An attempt was made to repeat published work, using a spectrophotofluorometric assay for biphenyl metabolites. The assay system, however, was not found to be valid for use with complex mixtures. Tests showed that metabolites must be separated from interfering compounds prior to quantitation.

A high pressure liquid chromatography method was developed to permit rapid separation of metabolites Nanogram quantities of metabolites were detectable by chromatographic separation in conjunction with a spectrophotofluorometric detector. It was not possible to demonstrate *in vitro* stimulation of biphenyl-2-hydroxylase by chemical carcinogens with this method.

In studies with alternative assays, terphenyl was metabolized to at least three different compounds by hamster microsomes. Further work is necessary to validate the utility of this substrate in an enzymatic screen for carcinogens.

A marine protozoan *(Parauronema acutum)* has been shown to metabolize biphenyl *in vivo* to 2- and 4-hydroxybiphenyl. This organism may provide a reliable, inexpensive source of biphenyl hydroxylase for an *in vitro* enzymatic assay system.

The effect of the addition of biphenyl on growth of *P. acutum* was examined at both 22° and 25°C. In addition, the effect of the carrier (dimethylsulfide [DMSO] or Tween 80) on the ability of the organism to respond to biphenyl was investigated.

At 22°C, biphenyl dissolved in DMSO at final concentrations in the culture of above 0.2 mM caused immediate death and lysis of the cells (Fig. 30). The lower concentration allowed normal growth of the culture in terms of cell counts. The loss in viability of the cultures was

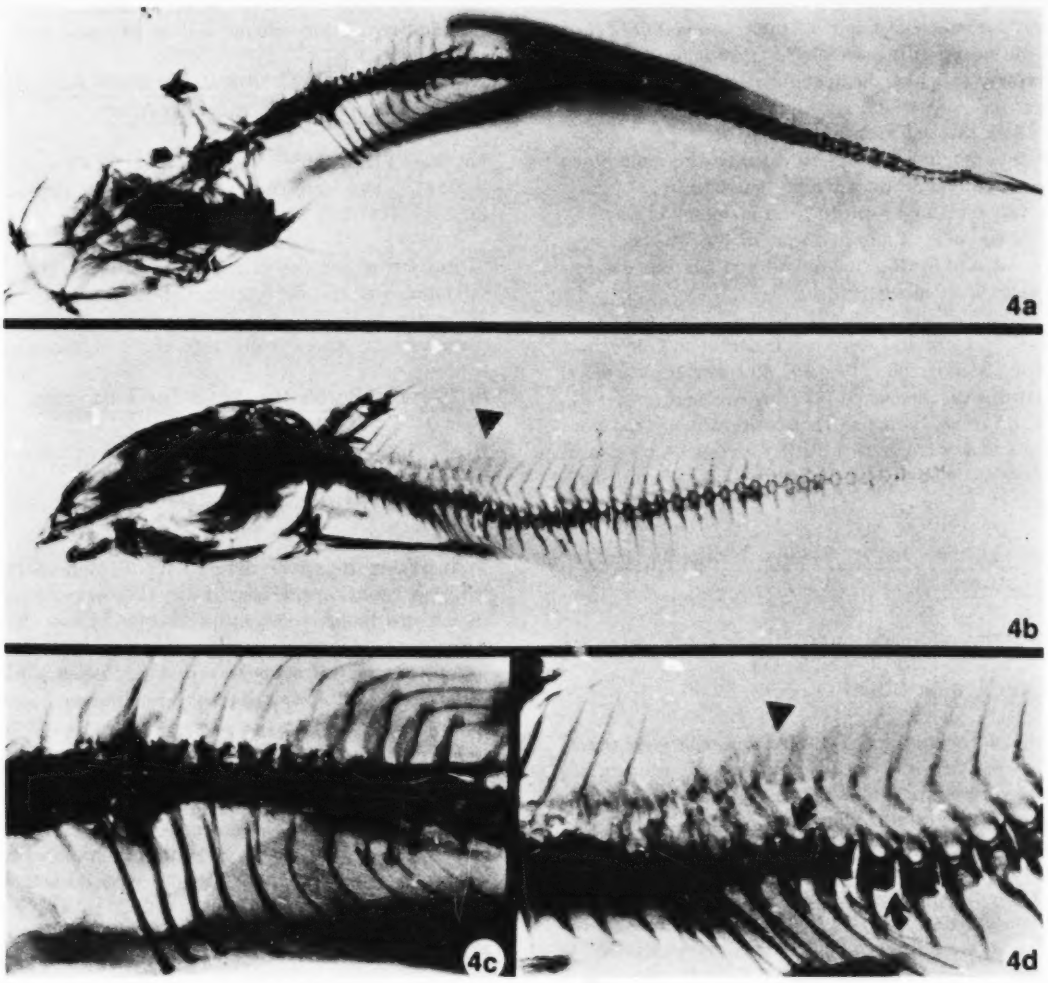

Figure 29. Vertebral disorientations in BaP-exposed catfish: (4a) dorsal view, Mag. 2X; (4b) lateral view, Mag. 2X; (4c) dorsal view, Mag. 5X; (4d) lateral view, Mag. 5X. Some vertebrae extend above adjacent vertebrae; others have abnormally large spacing (arrows). The rotation of some vertebrae removed their neural spines from the plane of the image (arrowhead).

caused by the biphenyl and not the DMSO carrier. However, when Tween 80 was the carrier, the lethal effect at higher concentrations of biphenyl was decreased.

At 25°C, there appeared to be no difference between biphenyl dissolved in DMSO or Tween 80, with cultures being unaffected by 0.2 mM concentrations of biphenyl (Figs. 31 and 32). Extracts of these cultures were examined by HPLC-SPF to quantitate the metabolites produced (Table 14). (Numbers are provided for those extracts in which metabolites were detected.)

It can be seen that both 2- and 4-hydroxybiphenyl were produced, and that neither carrier nor medium produced material which interfered with metabolite determination. When Tween 80 was used as a carrier, the results obtained seemed to indicate that the 4-hydroxybiphenyl metabolite may be located intracellularly in a form which is released by freezing and thawing the cells.

TABLE 14. QUANTITIES OF 2- AND 4-HYDROXYBIPHENYL PRESENT IN EXTRACTS OF *Parauronema acutum* CULTURES*

Additions to Incubation Mixture	Treatment Before Extraction	ng		Ratio 4-OH/ 2-OH
		2-hydroxybiphenyl	4-hydroxybiphenyl	
cells + medium**	None	-0-	-0-	- -
	Frozen	-0-	-0-	- -
0.2 mM biphenyl in DMSO (.01/10)***	None	0.43	1.26	2.9
	Frozen	0.44	0.98	2.2
1.0 mM biphenyl in DMSO (.05/10)	None	0.43	0.60	1.4
	Frozen	0.40	3.24	8.1
DMSO (.01/10)	None	-0-	1.06	- -
	Frozen	-0-	-0-	- -
DMSO (.05/10)	None	-0-	-0-	- -
	Frozen	0.10	-0-	- -
0.2 mM biphenyl in 1.5×10^{-2}% Tween[2]	None	0.75	1.38	1.8
	Frozen	0.80	3.12	3.9
1.0 mM biphenyl in 7.5×10^{-2}% Tween[2]	None	0.34	0.40	1.2
	Frozen	0.20	1.05	5.2
1.5×10^{-2}% Tween	None	-0-	-0-	- -
	Frozen	-0-	-0-	- -
7.5×10^{-2}% Tween	None	-0-	-0-	- -
	Frozen	0.35	0.45	- -

*Cultures were grown at 25°C. Growth data are presented in Figures 17 and 18.

**Numbers are the average obtained from two different culture flasks. All others represent one flask.

***Numbers in parentheses indicate the volume (ml) of biphenyl in DMSO or DMSO alone added to the 10 ml of medium.

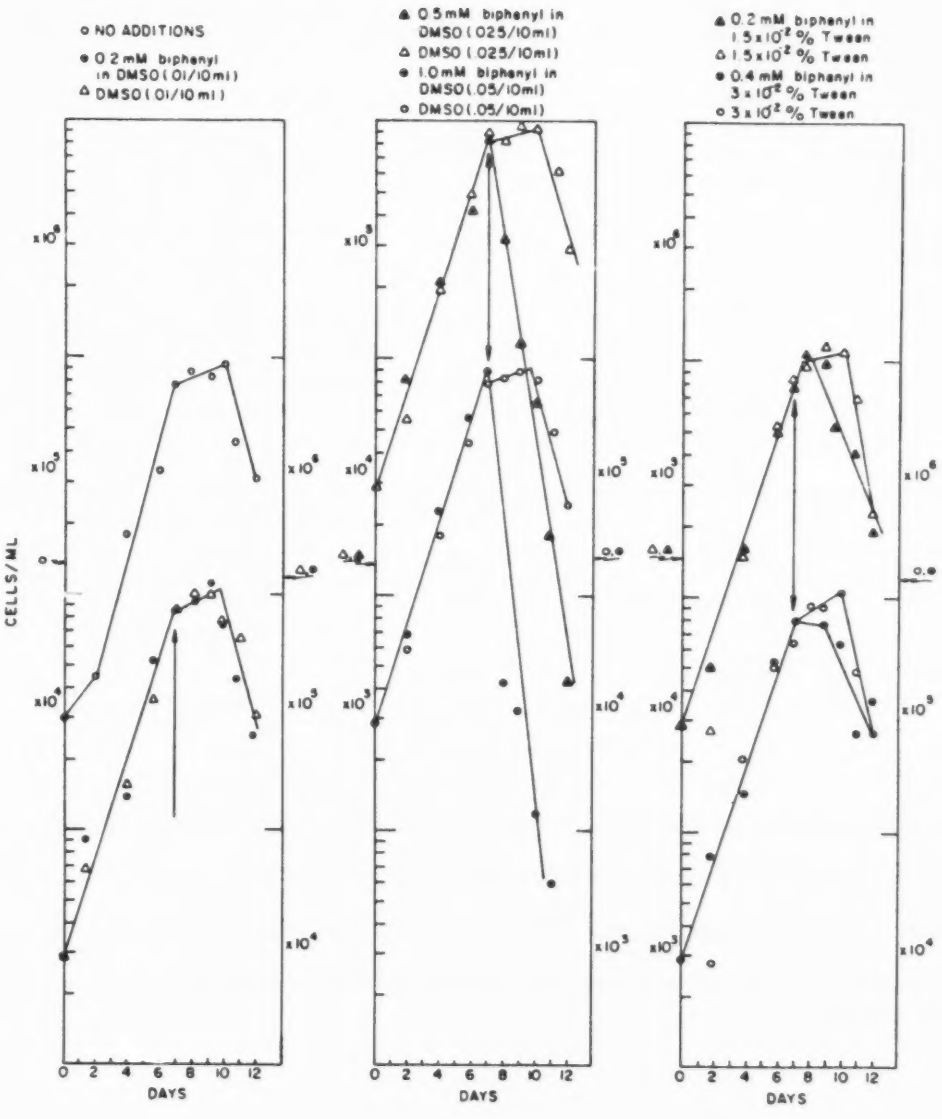
o NO ADDITIONS
• 0.2 mM biphenyl in DMSO (.01/10ml)
Δ DMSO (.01/10ml)
▲ 0.5 mM biphenyl in DMSO (.025/10ml)
Δ DMSO (.025/10ml)
• 1.0 mM biphenyl in DMSO (.05/10ml)
o DMSO (.05/10ml)
▲ 0.2 mM biphenyl in 1.5x10⁻² % Tween
Δ 1.5x10⁻² % Tween
• 0.4 mM biphenyl in 3x10⁻² % Tween
o 3x10⁻² % Tween
CELLS/ML
DAYS
0 2 4 6 8 10 12

Figure 30. Growth of Parauronema acutum *in the presence of biphenyl at 22°C. The arrows indicate time of addition of biphenyl or carrier (7 days), and each point is the mean of the cell counts from at least two different cultures in one experiment.*

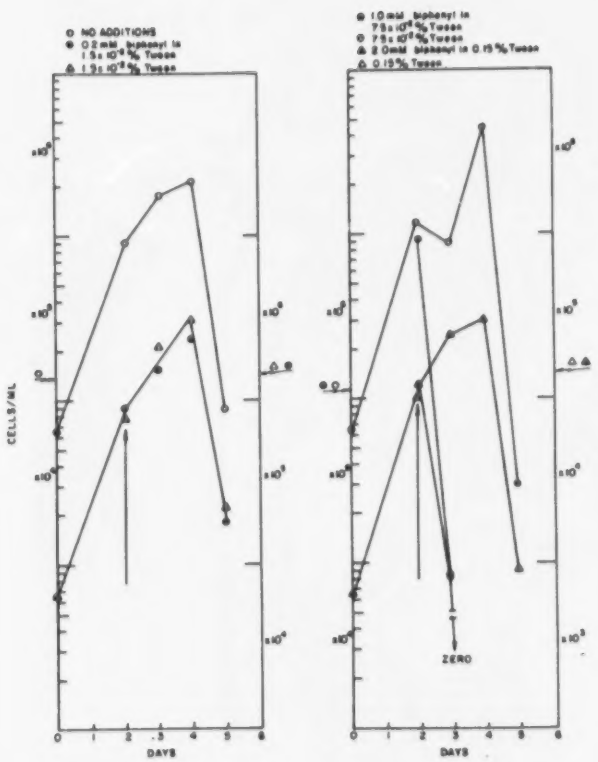

Figure 31. Growth of Parauronema acutum *at 25°C in the presence of biphenyl dissolved in DMSO. The arrows indicate time of addition of biphenyl or carrier (2 days), and each point is the mean of the cell counts from at least two different cultures in one experiment.*

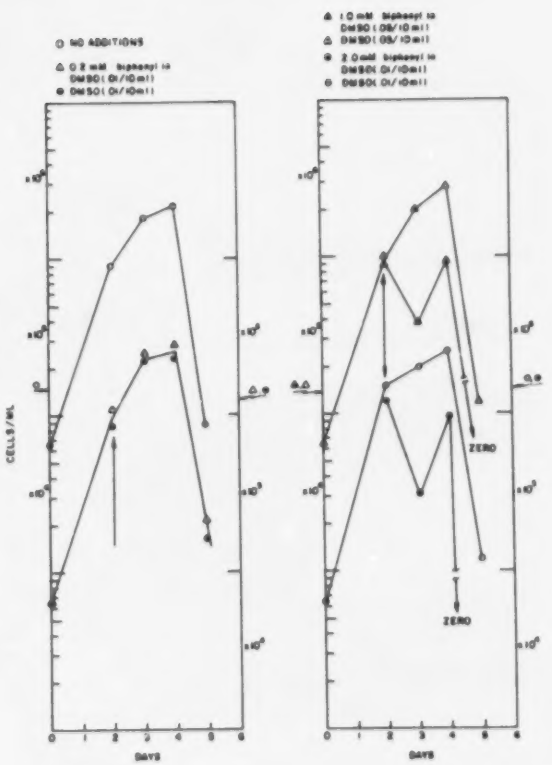

Figure 32. Growth of Parauronema acutum *at 25°C in the presence of biphenyl dissolved in Tween 80. The arrows indicate time of addition of biphenyl or carrier (2 days), and each point is the mean of the cell counts from at least two different cultures in one experiment.*

Figure 33. Biological Technician J.M. Patrick is assisted in securing full-face mask prior to diving for test samples from ERL,GB's offshore laboratory in the Gulf of Mexico.

EFFECTS OF OFFSHORE DRILLING FLUIDS ON THE MARINE ENVIRONMENT

The urgent demand for new sources of domestic oil and gas has brought about an intensified search for offshore petroleum. The number of "wildcat" or exploratory wells required to locate petroleum reserves has increased as the more accessible energy sources have become exhausted. Further, improved drilling technology has introduced a capability for drilling deeper wells.

Accelerated drilling activity in marine waters and deeper drilling practices have resulted in an increase in the quantity of chemicals being discharged into the marine environment. Many new chemicals have been developed for use in drilling fluids; some have not been tested for environmental effects.

ERL,GB's assessment of the potential impact of drilling fluids on the marine environment seeks to provide a data base for mitigating decisions regarding adverse effects. Drilling muds composed of diverse chemicals and released at varying underwater depths are evaluated on the basis of their effects on marine organisms and communities. Selected drilling fluid components are screened to determine their relative toxicity.

Data required for environmental impact statements, lease stipulations, discharge permits, and monitoring programs will be compiled from ERL,GB's investigation of:

(1) ecological impact of different classes of drilling fluids, cuttings, and packer fluids, including biocides (Fig. 33);

(2) advisability of banning or restricting the use of specific chemical additives;

(3) impact of drilling near areas of high biological productivity, such as coral reefs and bathometric highs.

Community Bioassays

M.E. TAGATZ, Research Aquatic Biologist;
J.M. IVEY, Biological Technician

Knowledge of the toxicity of biocides is required to assess their potential impact on the estuarine and marine environment. Communities of bottom-dwelling animals were used to investigate two biocides, Aldacide® (active component, paraformaldehyde) and Surflo® B33 (active component, sodium salt of 2, 2'-methylenebis), in laboratory tests at ERL,GB.

After analysis, results will be compared with data from earlier experiments using another biocide, pentachlorophenol (PCP). Initial findings indicate that paraformaldehyde was less toxic to macrobenthic communities used in experiments conducted at ERL,GB and in the field. The chlorophenols were found to be particularly toxic to mollusks.

Effects of Drilling Fluids and Oil on Corals

J.H. THOMPSON, Research Biologist; T.J. BRIGHT, Principal Investigators; EPA Grant R805441, Texas A&M Research Foundation, College Station, TX;
N.L. RICHARDS, Project Officer

Field experiments were conducted on *Stage I*, an offshore research platform leased from the U.S. Naval Coastal Systems Laboratory (Fig. 34), to determine the direct and indirect effects of drilling fluids on corals, coral communities, and coral reef processes. The platform, located 19 km south of Panama City, FL, in the Gulf of Mexico, provided field validations for laboratory tests to assess the potential of environmental hazards to coral reefs adjacent to offshore oil and gas drilling sites. Data relevant to the impact of chemicals discharged in offshore oil and gas drilling operations are required by EPA regulatory offices for decisions on applications for the National Pollutant Discharge Elimination System (N.P.D.E.S.) Permits.

The potential impact of effluents released near coral reefs during normal drilling activities has created concern for the biological, commercial, and aesthetic aspects of the reefs and the communities they shelter. In the 1978 experiments, *Madracis mirabilis* was selected as the test organism because of its significance to the Flower Gardens off the coast of Galveston, TX, and its behavioral patterns (expansion of polyps during the day and night that permit observation by time-lapse photography).

Coral samples were collected from the East Flower Garden banks in the western Gulf of Mexico and transported to the *Stage I* laboratory in tanks containing seawater (Fig. 35).

Previous work by the research team has indicated that the individual major sedimentary components of typical drilling fluids can be less harmful to coral colonies than the whole drilling mud taken from an actual drilling operation. Therefore, the hermatypic coral *(M. mirabilis)* was exposed to various lignosulfonates and chrome-modified lignosulfonates used as thinners in most drilling fluids. Chronic behavioral bioassays also were conducted in flowing seawater at *Stage I*. The coral also was exposed to various concentrations of ferrochrome lignosulfonate.

In separate experiments, colonies of *M. mirabilis* were subjected to similar concentrations of a whole drilling mud. Specially designed coral exposure chambers maintained a constant suspension of the drilling fluid in seawater. In both experiments, the behavior of the corals was measured with time-lapse movie cameras. Analysis of the movies permitted calculation of degree of polyp retraction: 100 ppm ferrochrome lignosulfonate (FCL) was 100% fatal to the coral after 5 days; 10 ppm FCL caused significant negative behavioral reaction as measured

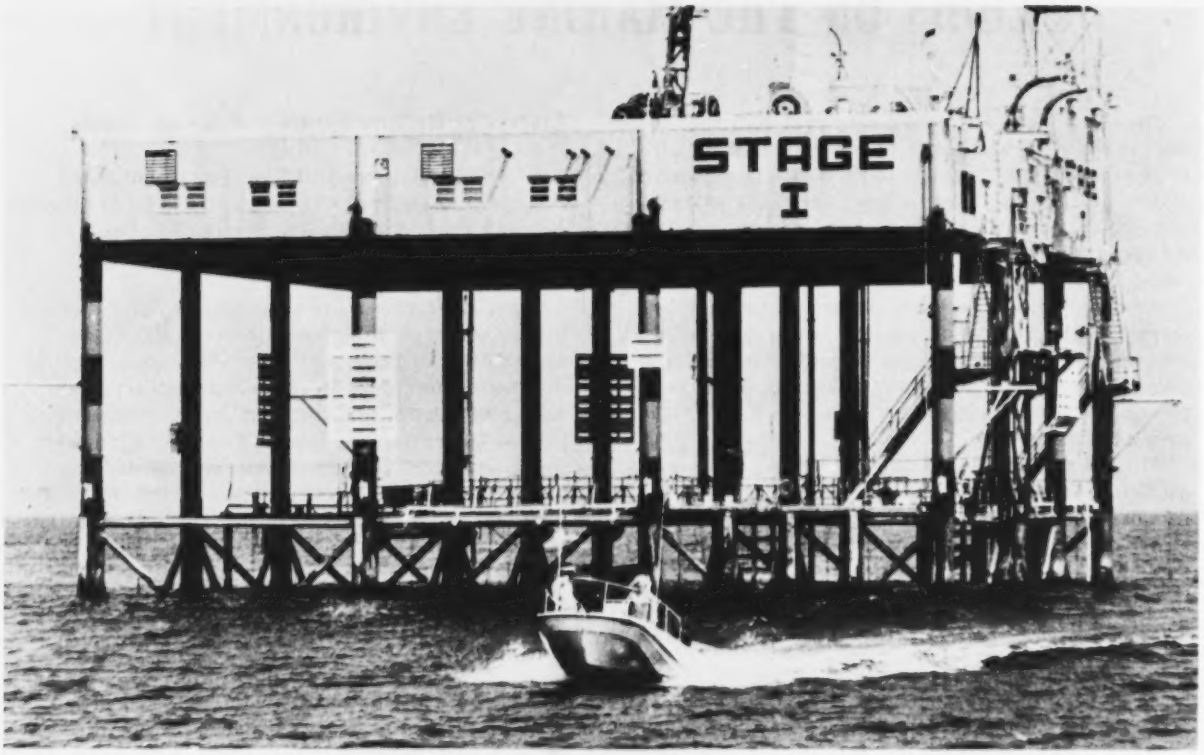

Figure 34. ERL,GB researchers head ashore from Stage I, *a U.S. Navy platform that serves as an offshore laboratory to test effects of compounds used in oil-drilling activities.*

by the R factor. Increased sedimentation and turbidity were observed. In the natural environment, these conditions are known to be deleterious to growth and recruitment rates of corals.

The results of chronic bioassays performed in flowing seawater on *M. mirabilis* indicate that some coral reef organisms were much more sensitive to FCL than were fish and other organisms used in earlier acute bioassays. Under some circumstances, concentrations of FCL capable of stressing *M. mirabilis* may be found more than 2 miles (3.2 km) from a drilling rig. Further dilution of an order of magnitude may not occur for 20 miles (32 km).

The effect of Q-Broxin on *M. mirabilis* was dramatic. Most corals treated with 100 ppm FCL retracted their polyps in about 1.6 hr. Although partial re-expansion occurred in some cases, all polyps (22 colonies) failed to re-expand after 19 hr exposure. After 120 hr (5 days), all corals in the 100 ppm treatment tanks went into the "shutdown reaction." (When this phenomenon occurs, the corals produce large amounts of mucus, often expel their zoozanthellae, and, within a few hours, their tissue disintegrates and decays, leaving a bare carbonate skeleton.)

As a result of preliminary observations, the ERL,GB coral effects program will be expanded. Drilling fluid components and whole drilling fluids will be tested at concentrations normally found near offshore oil and gas drilling operations to determine if these operations may pose a hazard to coral reefs.

Negative Ion Screening for Marine Xenobiotic Chemicals

R.C. DOUGHERTY, Principal Investigator; EPA Grant R806334, Florida State University, Tallahassee, FL; N.L. RICHARDS, Project Officer

Negative chemical ionization mass spectrometry (NCI-MS) is uniquely suited to the detection of toxic substances in the environment. This suitability stems from the fact that toxic substances generally have significant electron affinities and anion affinities. In contrast, biomolecules generally have negative electron affinities and attach anions only weakly in the gas phase.

Under an EPA grant, scientists examined a series of reagent gases to obtain a negative chemical ionization spectra of polynuclear aromatic hydrocarbons. With hydrocarbon or halocarbon reagent gases (i.e., isobutane or methylene chloride), the NCI mass spectrum is generally dominated by the molecular anion of the arene.

Sensitivity for many polynuclear aromatics increases significantly by the addition of 10% oxygen to a hydrocarbon reagent gas. The major ions in the spectra include

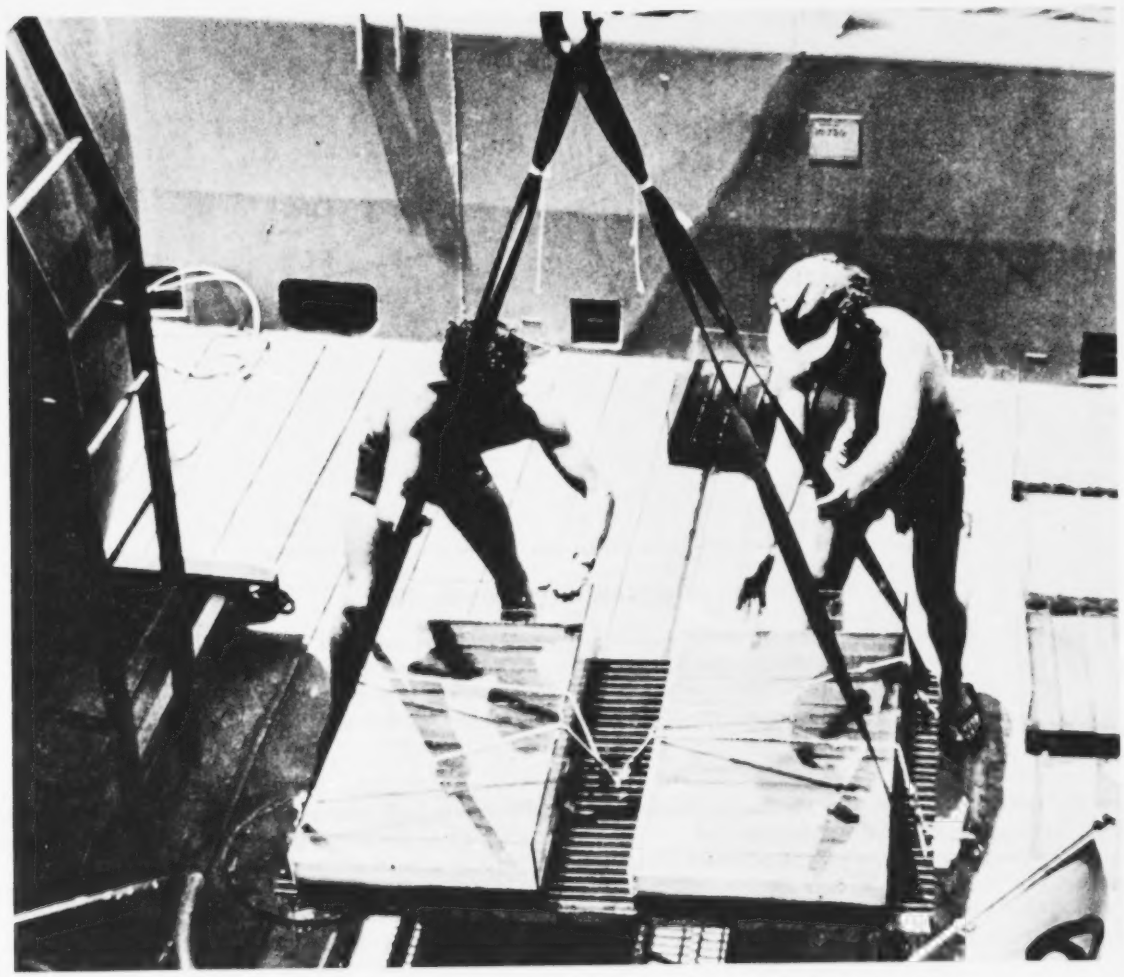

Figure 35. Stage I *Biological Aides C.M. Teaf and W.S. Ravenel steady tanks being lifted by crane into a landing barge for delivery to the offshore laboratory.*

the molecular anion, an oxygen exchange ion that corresponds to a deprotonated arene-ol, and oxygen aduct anions.

NCI mass spectra have been described for a series of polynuclear aromatic hydrocarbons. Sensitivity data and detection limits have been established for selected compounds in water.

Toxic, Sublethal, and Latent Effects of Selected Petroleum Hydrocarbons on Grass Shrimp

F.R. FOX, Research Biologist; EPA Grant R8044541,
University of West Florida, Pensacola, FL;
K.R. RAO, Principal Investigator;
N.L. RICHARDS, Project Officer

The short-term uptake, tissue distribution and depuration of two radio-labeled polycyclic aromatic hydrocarbons, benzo[a] pyrene (BaP) and benzanthracene (BA), were studied with the grass shrimp *(Palaemonetes pugio)* at known stages of the intermolt cycle.

Premolt shrimp accumulated less BaP and BA than intermolt shrimp. The newly molted shrimp accumulated more BaP and BA than intermolt shrimp. The relative increase in uptake by newly molted shrimp was more pronounced for BA than for BaP.

When exposed to 1.25, 2.5, 5, and 10 ppb BaP or BA, the intermolt shrimp accumulated BA to a greater extent than BaP at each of the concentrations tested.

The level of BA or BaP accumulated by shrimp increased in relation to the environmental levels of these compounds. The relative accumulation of BA and BaP in the tissues examined was in the following order: digestive tract (stomach + intestine) hepatopancreas thorax abdomen. Each tissue accumulated more BA than BaP.

A rapid uptake was observed in shrimp exposed to media containing 2.5 ppb BA or BaP during the first 6 hr; subsequently uptake was somewhat reduced. However,

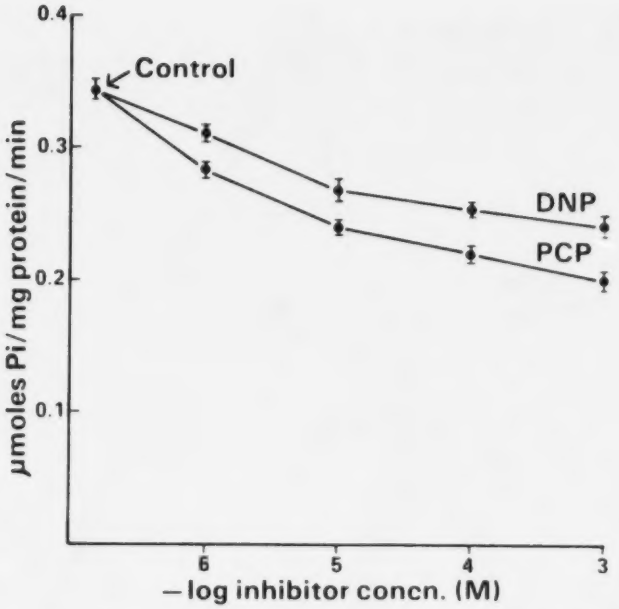

Figure 36. In vitro *effects of sodium pentachlorophenate and 2,4-dinitrophenol on a calcium-activated ATPase in the microsomal fraction of the hepatopancreas from the blue crab* (Callinectes sapidus). *The values are mean ± S. D. of five experiments. (Reprinted by permission of Plenum Publishing Corp., New York.)*

even at the end of 96 hr exposure, the shrimp exhibited a trend of continual accumulation of BA and BaP.

When transferred to seawater, the shrimp appeared to depurate BA more rapidly than BaP. The level of radioactivity in the shrimp exposed to BA declined by 80% at the end of a 7-day period of depuration; under similar conditions the BaP level (radioactivity) declined by only 35%.

Toxicity of Pentachlorophenol to Crustaceans

K.R. RAO, Principal Investigator; EPA Grant R8044541, University of West Florida, Pensacola, FL;
N.L. RICHARDS, Project Officer

Crustaceans are capable of regenerating lost limbs. The incidence of limb regeneration, the rate of growth of the limb bud and the relative size of the new limb after ecdysis depend on (1) the stage of the molt cycle at which limb removal occurs and (2) the interval between limb removal and ecdysis.

Exposure to media containing sodium pentachlorophenate (Na-PCP) caused a dose-related inhibition of limb regeneration in grass shrimp *(Palaemonetes pugio).* The early phases of regeneration (wound healing, cell division, and dedifferentiation) appeared to be more sensitive to Na-PCP than later phases of regenerations.

The effects of Na-PCP on oxygen consumption by the grass shrimp varied depending on the stage of the molt cycle and the concentration of Na-PCP. Intermolt shrimp exposed to high concentrations of Na-PCP (10 or 20 ppm) exhibited an initial increase in oxygen consumption followed by a rapid decline, leading to death.

A decline in oxygen consumption followed by death can be induced in newly molted shrimp by a lower concentration (5 ppm) of Na-PCP. Tests on isolated tissues (muscle, gill, and hepatopancreas) from the blue crab *(Callinectes sapidus)* revealed that Na-PCP and 2,4-dinitrophenol (DNP) inhibit oxygen consumption *in vitro.*

Na-PCP and DNP had inhibitory effects on several hepatopancreatic enzymes in blue crabs. The enzymes affected were: fumarase, succinate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, pyruvate kinase, lactic dehydrogenase, glutamate-pyruvate transaminase and a microsomal calcium-activated ATPase. Under *in vitro* conditions, isocitrate dehydrogenase was stimulated by a low concentration of Na-PCP (10^{-6}M) whereas higher concentrations inhibited it.

These studies on crustaceans and earlier studies on fish, mollusks, and rats seem to indicate that PCP affects carbohydrate metabolism, lipid metabolism, ion transport, and possibly protein metabolism. The inhibitory effects on a wide variety of enzymes suggest that the actions may be due to non-specific interactions of this phenol with membrane proteins.

In addition to the proven uncoupling effects on oxidative phosphorylation, the overall inhibitory effects of PCP on a variety of enzymes (Fig. 36) may account for the broad-spectrum biocidal effects of pentachlorophenol (PCP) and its salts.

Toxic Photooxygenated Products Generated Under Environmental Conditions from Phenanthrene

J.L. LASETER, Principal Investigator; EPA Grant R804647, University of New Orleans, LA; N.L. RICHARDS, Project Officer

The photooxidation of phenanthrene, a model polycyclic aromatic hydrocarbon (PAH), was accomplished in a hexane aqueous phase. A light source similar to sunlight was used to simulate environmental conditions.

The involvement of singlet oxygen also was examined. Several oxygenated toxic photoproducts were characterized by a gas chromatograph-mass spectrometer computer system. Some products were found to be soluble in water, suggesting the possibility of oxygenated PAH intrusion into natural waters.

Generic Variation and Resistance to Carcinogens in Natural Waters

R.J. SCHULTZ, Principal Investigator; EPA Grant R805195, University of Connecticut, Storrs, CT; N.L. RICHARDS, Project Officer

When test species are selected to investigate the toxic or carcinogenic properties of pollutants, selection criteria are usually influenced by availability and adaptability to the laboratory. A third consideration–the genetic background of the indicator species–is being evaluated in tests that will compare the resistance of fish of different stocks to dimethylbenzanthracene and diethylinitrosamine.

Novel Techniques for Concentration and Separation of Toxic Substances from Estuarine Waters

E. KLEIN, Principal Investigator; EPA Grant R805656, Gulf South Research Institute, New Orleans, LA; N.L. RICHARDS, Project Officer.

The organic carcinogens benzo[a] pyrene (BaP), dieldrin, and N-acetyl-2-amino-fluorene were recovered on XAD-2 macroreticular resin in yields of 90% or more from distilled water or seawater and in yields of 40% or more from Lake Pontchartrain water containing a high concentration of organic material.

The original solutions contained less than 500 parts per trillion (ppt) of carcinogen. These results show that XAD-2 provides an efficient means for recovering nonpolar organic carcinogens from dilute solutions.

More polar carcinogens such as diethylinitrosamine were not effectively recovered on XAD-2 columns. Therefore, the ability of the above carcinogens to bind to nucleic acid both before and after S9 liver microsomal activation was investigated.

The DNA-carcinogen interaction systems included direct binding, equilibrium dialysis, nuclei binding, and binding to DNA-cellulose. Radiolabeled carcinogens were used to quantify the amount bound.

Either rat liver nuclei (0.1 mg DNA) or DNA-cellulose (1 mg DNA) bound 18% of the acetylaminofluorene and up to 66% of the dieldrin from solutions containing 150 to 280 nmoles of compound. Up to 30% from solutions containing as much as 320 pmoles was bound. Ten-fold or lower recoveries were found when direct-binding or equilibrium-binding methods were used.

Less carcinogen was recovered with the DNA fraction when liver microsomes were used to activate the binding systems. In these cases, the microsomal protein can decrease the net DNA binding by competing with the DNA for carcinogen or by converting the carcinogen to products that do not bind well to DNA.

In the nuclei binding studies, DNA was isolated from the nuclei by extraction with sodium dodecyl sulfate and pehnol followed by recovery of the DNA by ethanol precipitation and spooling. The recovered DNA contained more than 10% of the input radioactivity, indicating that a significant portion of the input sample may be tightly bound to DNA.

The portion that bound after DNA isolation was not dissociated by further extractions with chloroform-isoamyl alcohol or 1% sodium dodecyl sulfate solution. At least some of this material may be covalently associated. Whether or not DNA-binding is more specific for recovery of organic carcinogens than is XAD-2 has not been determined.

Activation of 2-aminofluorene to Mutagen(s) by the Marine Ciliate *(Parauronema acutum)*

D.C. LINDMARK, Principal Investigator; EPA Grant R805364, Rockefeller University, New York, NY; N.L. RICHARDS, Project Officer

Living cells of the marine ciliate *(Parauronema acutum)* can convert 2-aminofluorene into compounds with mutagenic activity in the Ames/*Salmonella* plating test using tester strains TA 98, 1537, 1538. The ciliate, however, does not activate benzo[a] pyrene or destroy the mutagenic properties of N-methyl-N'-nitro-N-nitrosoguanidine, and does not accumulate any of the tested compounds.

Homogenates of *P. acutum,* when substituted for a liver microsomal fraction in the *Salmonella*/microsome test, activate 2-aminofluorene and produce revertants at a high rate. Benzo[a] pyrene is not activated nor is nitrosoguanidine inactivated.

After differential sedimentation of an homogenate, the activating ability is equally distributed between a non-sedimentable fraction and a fraction sedimenting at high speed. Though the activation of aminofluorene does not require NADP and may not be due to mixed function oxidase activity, it does demonstrate that a marine ciliate can produce mutagens and can possibly contribute to an increase of the mutagen load in the marine environment.

The role of marine protozoa in the accumulation of polynuclear aromatic hydrocarbons (PAH) will be assessed. Microsomal fractions were prepared in 1978 and the microsomal activity of marine protozoa will be characterized in 1979.

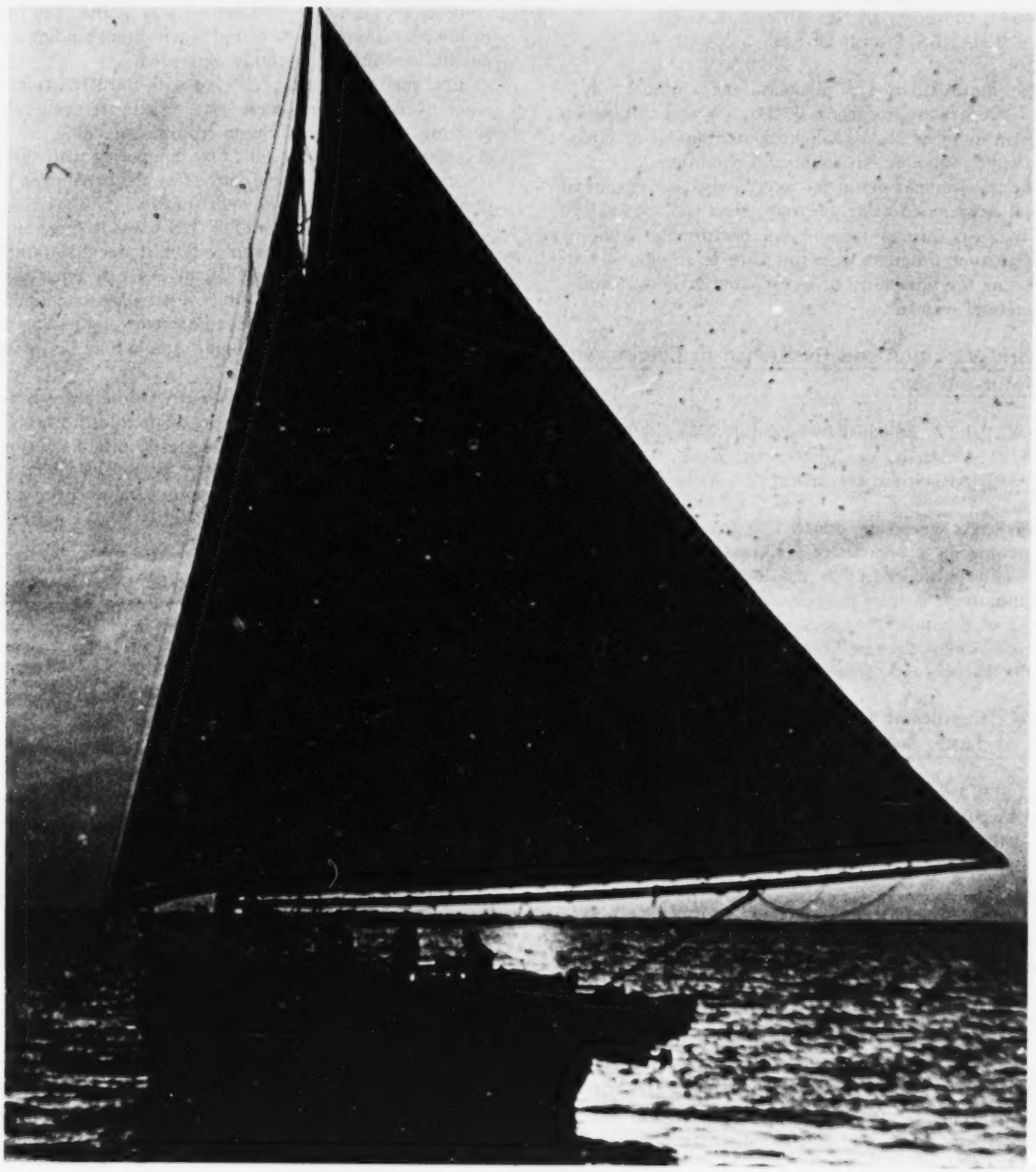

Figure 37. The Chesapeake Bay (above), a popular site for water recreation, is the site of an EPA research effort designed to restore and preserve the quality of natural systems of the Bay estuary.

CHESAPEAKE BAY PROGRAM

In the independent Agencies Appropriation Bill of 1976, the 94th Congress directed EPA to assess adverse environmental impacts on the Chesapeake Bay system and to coordinate a research/abatement program aimed at restoring the quality of the estuary.

In response, EPA established the Chesapeake Bay Program (CBP) and joined with environmental agencies of the states within the Bay drainage basin, interested scientists, and private citizens in an attempt to define and establish pollution problem priorities and to recommend measures for the proper management of Bay resources.

ERL, Gulf Breeze Deputy Director T.T. Davies is director of CBP's varied and interdependent scientific and management investigations of the Chesapeake Bay System. Results of the research will establish a framework to identify problems, describe processes affected, and project consequences of identified control measures in both the natural and management systems.

In fostering an atmosphere of partnership in CBP, funds were awarded to the water pollution control agencies in Maryland and Virginia and to an "umbrella" organization of citizen interests. The State management grants allow coordinated channeling of all State concerns through one designated lead agency and enhance the communication between the EPA program and each State. The public particpation program fosters development of communication channels between citizens and the CBP management and informs the public about the program and its objectives.

At the outset, three priority areas were identified for scientific investigations: (1) accumulation of toxicants in the food chain, (2) the decline of submerged aquatic vegetation, and (3) eutrophication (nutrient enrichment).

An evaluation of the role of toxics in the ecological health of the Chesapeake Bay system requires a thorough understanding of the chemical, physical, and biotic dynamics of the estuarine system. The CBP toxics program is concerned with the sources, pathways, and fate of toxic substances present in the estuary. Initial toxic studies will develop a baseline inventory of the distribution of toxicants in sediments, pore water, the water column, and biota. Results of the toxics program will be used to delineate management options to control toxicants and minimize their adverse impacts.

Submerged Aquatic Vegetation (SAV) Program will examine the cause-and-effect relationships potentially responsible for the decline of bottom grasses in the Bay system. Studies are designed to identify, and, when possible, to quantify important functions performed by SAV as a source for food, shelter, and habitats and breeding areas for finfish and shellfish, water fowl, and species of the lower trophic levels. Research results will provide data for a management plan aimed at protecting and enhancing the growth and propagation of the Bay's submerged plants.

Eutrophication studies traditionally focus on the superabundant nutrients that promote excessive algal growth, subsequent depletion of dissolved oxygen, and an eventual imbalance of the ecosystem. To date, very little scientific data are available on the liabilities and benefits of nutrient enrichment in an estuarine environment. The Eutrophication Program will address the question of nutrient enrichment in the estuary. Additionally, intensive watershed and modeling studies will investigate in-stream and ecosystem responses to non-point source loadings. An understanding of the eutrophication and enrichment processes will aid Bay managers in evaluating the consequences of possible control alternatives and developing control priorities.

The scientific and technical investigations undertaken by the Chesapeake Bay Program are designed to provide products and data targeted toward improved management of the Bay and its resources. An Environmental Quality Management Study (EQMS) will survey and describe current Bay management institutions and agencies, review available water resource management alternatives, and define institutional strategies to implement improved Bay management. Socio-economic costs and benefits related to various levels of water quality and abatement control alternatives also will be identified within the EQMS effort.

Chesapeake Bay research projects are listed below:

TOXICS

Chesapeake Bay Earth Science Study--Sedimentology of the Chesapeake Bay

R.T. KERHIN, Principapl Investigator;
EPA Grant R805965, Maryland Geological Survey, Baltimore, MD;
L.H. BAHNER, Project Officer

Baseline Sediment Studies to Determine Distribution, Physical Properties, Sedimentation Budget, and Rates

J.M. ZIEGLER, Principal Investigator;
EPA Grant R806001, Virginia Institute of Marine Sciences, Gloucester Point, VA;
L.H. BAHNER, Project Officer

Investigation of Organic Pollutants in the Chesapeake Bay

R.J. HUGGETT, Principal Investigator;
EPA Grant R806012, Virginia Institute of Marine Sciences, Gloucester Point, VA;
L.H. BAHNER, Project Officer

Chesapeake Bay Earth Science Study - Interstitial Water Chemistry

O.P. BRICKER, Principal Investigator;
EPA Grant R805963, Maryland Geological Survey, Baltimore, MD;
L.H. BAHNER, Project Officer

The Biogenic Structure of Chesapeake Bay Sediments

D.F. BOESCH, Principal Investigator;
EPA Grant R805982, Virginia Institute of Marine Sciences, Gloucester Point, VA;
L.H. BAHNER, Project Officer

Chesapeake Bay Sediment Trace Metals

G.R. HELZ, Principal Investigator;
EPA Grant R805954, University of Maryland, College Park, MD;
L.H. BAHNER, Project Officer

Monitoring Particle-associated Toxic Substances and Suspended Sediment in the Chesapeake Bay

W. TAYLOR, Principal Investigator;
EPA Grant R805959, The Johns Hopkins University Chesapeake Bay Institute, Baltimore, MD;
L.H. BAHNER, Project Officer

Fate, Transport, and Transformation of Toxics: Significance of Suspended Sediment and Fluid Mud

M. NICHOLS, Principal Investigator;
EPA Grant R806002, Virginia Institute of Marine Sciences, Gloucester Point, VA;
L.H. BAHNER, Project Officer

Chesapeake Bay Earth Science Study - Animal Sediment Relationship

O.P. BRICKER, Principal Investigator;
EPA Grant R805964, Maryland Geological Survey, Baltimore, MD;
L.H. BAHNER, Project Officer

Sediment and Pore Water Chemistry

S.Y. TYREE, Jr., Principal Investigator;
EPA Grant R805966, College of William and Mary, Williamsburg, VA;
L.H. BAHNER, Project Officer

The Characterization of the Chesapeake Bay: A Systematic Analysis of Toxic Trace Elements

C.C. GRAVETT, Principal Investigator; Interagency Agreement EPA-79-D-X0717, National Bureau of Standards, Washington, DC;
L.H. BAHNER, Project Officer

Investigation of the Chester River Oyster Mortality

H. WILSON, Principal Investigator;
EPA Grant R805976, Water Resources Administration, Maryland Department of Natural Resources, Annapolis, MD;
L.H. BAHNER, Project Officer

SUBMERGED AQUATIC VEGETATION (SAV)

Distribution of Submerged Vascular Plants in the Chesapeake Bay, Maryland--1978

R.R. ANDERSON, Principal Investigator;
EPA Grant R805977, The American University, Washington, DC;
W. COOK, Project Officer

Distribution and Abundance of SAV in the Chesapeake Bay--1978

R.J. ORTH, Principal Investigator;
EPA Grant R805951, Virginia Institute of Marine Sciences, Gloucester Point, VA;
W. COOK, Project Officer

Distribution and Abundance of SAV in the Lower Chesapeake Bay--1979

R.J. ORTH, Principal Investigator;
Project X-003201-01, Virginia Institute of Marine Sciences, Gloucester Point, VA;
T. NUGENT, Project Officer

Distribution of SAV in Chesapeake Bay, Maryland--1979

R.J. MACOMBER, Principal Investigator;
Project X-003202-01, Chesapeake Bay Foundation, Annapolis, MD;
T. NUGENT, Project Officer

Biostratigraphy of the Chesapeake Bay: A Feasibility Study

G.S. BRUSH, Principal Investigator;
EPA Grant R805962, Department of Geography and Environmental Engineering, The Johns Hopkins University, Baltimore, MD;
T. NUGENT, Project Officer

Biostratigraphy of the Chesapeake Bay and its Tributaries

G.S. BRUSH, Principal Investigator;
EPA Grant R806680, Department of Geography and Environmental Engineering, The Johns Hopkins University, Baltimore, MD;
T. NUGENT, Project Officer

The Functional Ecology of Submerged Aquatic Vegetation in the Lower Chesapeake Bay

R.L. WETZEL, Principal Investigator;
EPA Grant R805974, Virginia Institute of Marine Sciences, Gloucester Point, VA;
T. NUGENT, Project Officer

Submerged Aquatic Vegetation in the Chesapeake Bay: Its Role in the Bay Ecosystem and Factors Leading to its Decline

J.C. STEVENSON, Principal Investigator;
EPA Grant R805932, University of Maryland, Center for Environmental and Estuarine Studies, Horn Point Environmental Laboratories, Cambridge, MD;
T. NUGENT, Project Officer

Relationship Between Submerged Aquatic Vegetation and Waterfowl: Criteria and Techniques

F. MARTIN, Principal Investigator;
Interagency Agreement EPA-78-D-X-391, Migration Bird and Habitat Research Laboratory, U.S. Fish and Wildlife Service, Laurel, MD;
T. NUGENT, Project Officer

Studies on the Value of Vegetated Habitats and their Roles as Nursery Areas and Shelter from Predation with Emphasis on Utilization by Commercially Exploited Species

K.L. HECK, Jr., Principal Investigator;
EPA Grant R806151, Benedict Estuarine Research Laboratory, Benedict, MD;
T. NUGENT, Project Officer

Assessment of the Potential Impact of Industrial Effluents on Submerged Aquatic Vegetation

G.E. WALSH, Principal Investigator;
U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL;
T. NUGENT, Project Coordinator

Zostera Marina: Biology, Propagation, and Impact of Herbicides

R.J. ORTH, Principal Investigator;
EPA Grant R805953, Virginia Institute of Marine Sciences, Gloucester Point, VA;
T. NUGENT, Project Officer

Effects of Recreational Boating on Turbidity and Sedimentation Rates in Relationship to Submerged Aquatic Vegetation

J. WILLIAMS, H. GUCINSKI, Principal Investigators;
Interagency Agreement EPA-78-D-X-426, Department of Oceanography, U.S. Naval Academy, Annapolis, MD;
T. NUGENT, Project Officer

EUTROPHICATION

Definition of Chesapeake Bay Problems of Excessive Enrichment or Eutrophication

L.E. CRONIN, Principal Investigator;
EPA Grant R806189, Chesapeake Research Consortium, Annapolis, MD;
T. PHEIFFER, Project Officer

An Assessment of Nonpoint Source Discharge, Pequea Creek Basin, Lancaster County, Pennsylvania

R.J. BIELO, Principal Investigator;
Project X-003146-02, Susquehanna River Basin Commission, Harrisburg, PA;
T. PHEIFFER, Project Officer

Fall Line Monitoring of the Potomac, Susquehanna, and James Rivers

F. WHITE, Principal Investigator;
Interagency Agreement EPA-78-D-X-420, U.S. Geological Survey, Water Resources Division, Towson, MD;
T. PHEIFFER, Project Officer

Chesapeake Bay Circulation Model

R. SHUBINSKI, Principal Investigator;
Project 68-01-5125, Water Resources Engineers, Inc., Springfield, VA;
T. PHEIFFER, Project Officer

Evaluation of Water Quality Management Tools in the Chester River Basin

H. WILSON, Principal Investigator;
EPA Grant R806343, Water Resources Administration, Maryland Department of Natural Resources, Annapolis, MD;
T. PHEIFFER, Project Officer

Intensive Watershed Study (Patuxent River Basin)

H. WILSON, Principal Investigator;
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Evaluation of Management Tools in Two Chesapeake Bay Watersheds in Virginia

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Assessment of Nutrients from Various Sources

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Modeling Philosophy and Approach for Chesapeake Bay Program Watershed Studies

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Water Quality Laboratory for Chesapeake Bay and Its Subestuaries at Hampton Institute

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EPA Grant R806229, Department of Chemistry and Physics, Hampton Institute, Hampton, VA;
T. NUGENT, Project Officer

Land Use and Point Source Nutrient Loading in the Chesapeake Bay Region

B.J. MASON, Principal Investigator;
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ENVIRONMENTAL QUALITY MANAGEMENT

Preparation of a Strategy and Plan of Action for Designing the Research of Management Resources for the Chesapeake Bay Area

J. KEENE, Principal Investigator;
Project X-003149-01, Department of City and Regional Planning, University of Pennsylvania, Philadelphia, PA;
G. MCGINTY, Project Officer

Environmental Management in the Chesapeake Bay

R. HARRISON, Principal Investigator;
Project X-003200-01, Environmental Law Institute, Washington, DC;
G. MCGINTY, Project Officer

PUBLIC PARTICIPATION

Chesapeake Bay Program's Public Participation Program

F. FLANAGAN, G.M. HAGERMAN, Principal Investigators;
Project T-9008748-01 and T-9008790-01, Citizens Program for the Chesapeake Bay, Inc., Baltimore, MD;
W. COOK, Project Officer

STATE PARTICIPATION

Development and Coordination of Technical Assessments, Scientific Planning, and Data Organization for the Chesapeake Bay Program

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EPA Grant R805874, Water Resources Administration, Maryland Department of Natural Resources, Annapolis, MD;
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Data Organization, Technical Support, and Coordination for the Environmental Protection Agency's Chesapeake Bay Program

R. DAVIS, Principal Investigator;
EPA Grant R805859, Virginia State Water Control Board, Richmond, VA;
G. MCGINTY, Project Officer

PUBLICATIONS

Recent publications by the ERL,GB and Bears Bluff Field Station personnel and by researchers supported by EPA grants or contracts are listed below. Limited reprints are available and may be obtained from Betty Jackson, Technical Information Coordinator, Environmental Research Laboratory, Gulf Breeze, FL 32561, or through the National Technical Information Service, Springfield, VA 22161.

Research Reports

Anderson, Robert S. 1978. BENZO[a]PYRENE METABOLISM IN THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA*. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-78-009. 19 p.

This research program focuses on the role of NADPH-dependent microsomal mono-oxygenase in the metabolism of the widespread environmental carcinogen benzo[a]pyrene (BP) by the oyster *(Crassostrea virginica)*. The enzyme system is important in detoxifying various xenobiotics and in activating polycyclic aromatic hydrocarbon oncogens as BP.

A sensitive radioisotopic system developed to permit quantification of alkalid-soluble and water-soluble BP metabolites produced by oyster mono-oxygenase is described. An NADPH- and O_2-dependent aryl hydrocarbon hydroxylase (AHH) is shown to be located in the digestive glands of bivalves associated with the microsomal subcellular fraction. Some indication that oyster AHH is induced by chronic exposure of the animals to the environmental carcinogens BP and 3-methyl-cholanthrene is reported. Experimental evidence indicates that exposure to polychlorinated biphenyls (PCB) caused AHH induction. The generation of various dihydrodiol, quinone, and hydroxy BP derivates is shown.

Bierman, Victor, William Richardson, and Tudor T. Davies. 1978. MATHEMATICAL MODELING STRATEGIES APPLIED TO SAGINAW BAY, LAKE HURON. In: American-Soviet Symposium on Use of Mathematical Models to Optimize Water Quality Management, T.T. Davies and V.R. Lozanskiy, editors. Environmental Research Laboratory, Gulf Breeze, FL. EPA Ecological Research Series, EPA-600/9-78-024. pp. 397-432.

This research is directed toward water quality problems of international waters of the North American Great Lakes. The prime objective is to develop quantitative tools to supplement intuition and scientific judgment in policy decisions related to water quality. Transport models and algal growth modeling concepts are applied to Saginaw Bay to describe prevailing conditions.

Borthwick, Patrick W. 1978. METHODS FOR ACUTE STATIC TOXICITY TESTS WITH MYSID SHRIMP *(MYSIDOPSIS BAHIA)*. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 61-63.

Methods are described for using the bay mysid *(Mysidopsis bahia)* in acute toxicity tests of complex wastes. *M. bahia* is recommended as a test species due to its sensitivity, short life cycle, small size, and adaptability to laboratory conditions. Results of these toxicity tests can be used to estimate the impact of ocean-dumped materials on other saltwater crustaceans.

Bourquin, A.W., and P.H. Pritchard. 1979. PROCEEDINGS OF THE WORKSHOP: MICROBIAL DEGRADATION OF POLLUTANTS IN MARINE ENVIRONMENTS. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-79-012. 551 p.

This international workshop, held April 10-14, 1978, at Pensacola Beach, Florida, focuses on pertinent issues related to the scientific investigation of microbial degradation of organic chemicals in aquatic environments. Participants discuss methodological criteria for these investigations and the need for biodegradation studies. Speakers and contributed papers for open sessions explore these topics: (1) biochemistry of microbial degradation; (2) transformation in aquatic environments; (3) compartmentalization in aquatic environments; (4) biodegradation in microcosms; (5) degradation methodology; and (6) persistence and extrapolation. Discussions within each session are presented. These proceedings conclude with a summary report and workshop consensus reports drafted by special task groups with recommendations concerning the research, production, and regulation of potential aquatic pollutants.

Butler, P.A., and J.I. Lowe. 1978. FLOWING SEAWATER TOXICITY TEST USING OYSTERS *(CRASSOSTREA VIRGINICA)*. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 25-27.

A "special bioassay" for evaluating short-term effects of specific wastes on marine mollusks is described. The procedure is recommended only for use with the commercial Eastern oyster *(Crassostrea virginica)* and requires flowing, unfiltered seawater. The test is used at the Environmental Research Laboratory, Gulf Breeze, to evaluate the effects of insecticides, herbicides, and other toxic organics on oysters.

Cross, F.A., W.P. Davis, D.E. Hoss, and D.A. Wolfe. 1978. BIOLOGICAL OBSERVATIONS. In: The Amoco Cadiz Oil Spill: A Preliminary Scientific Report, Wilmot N. Hess, editor. U.S. Department of Commerce National

Oceanic and Atmospheric Administration (NOAA)/ Environmental Protection Agency (EPA) Special Report, Government Printing Office (GPO), Washington, DC. pp. 197-215.

This report is a compilation of observations and data gathered along the Brittany Coast of France after the *Amoco Cadiz* oil spill. The information does not reflect results of a pre-planned biological study, but rather the qualitative observations by NOAA/EPA biologists from late March to May 1978. The material is described as preliminary; final assessment of the full extent of the impact is expected to require several years.

Davies, T.T., and V.R. Lozanskiy, editors. 1978. AMERICAN-SOVIET SYMPOSIUM ON USE OF MATHEMATICAL MODELS TO OPTIMIZE WATER QUALITY MANAGEMENT. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-024. 453 p.

The American-Soviet Symposium on Use of Mathematical Models to Optimize Water Quality Management examines methodological questions related to simulation and optimization modeling of processes that determine water quality of river basins. Participants describe the general state of development and application of mathematical models designed to predict and optimize water quality management in the USA and USSR. American and Soviet specialists discuss graphic-economic aspects of pollution control systems; identification of ecosystem models by field data; management decisions for lake systems on a survey of trophic status, limiting nutrients, and nutrient loadings; and a descriptive simulation model for forecasting the condition of a water system. Publication of the proceedings held December 9-16, 1975, in Kharkov and Rostov-on-Don, USSR, is in compliance with the Memorandum from the Fourth Session of the Joint American-Soviet Committee on Cooperation in the Field of Environmental Research.

Duke, Thomas W., and Anatoliy I. Simonov, editors. 1978. FIRST AMERICAN-SOVIET SYMPOSIUM ON THE BIOLOGICAL EFFECTS OF POLLUTION ON MARINE ORGANISMS. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-007. 166 p.

American and Soviet specialists discuss state-of-the-art for hydrobiological analysis of basic structural components of marine ecosystems and the influence of various pollutants on these components. Participants define problems related to methods for modeling the influence of pollutants on the marine environment, long-term forecasting and determination of permissible loads of pollutants, and the unification and intercalibration of methods for determining production of microorganisms of ocean bacterioplankton and phytoplankton. Results of laboratory research on the influence of pollution on the marine environment are presented. Proceedings held September 20-24, 1976, in Gulf Breeze, FL, were published in English and Russian in compliance with the Memorandum from the Fourth Session of the Joint American-Soviet Committee on Cooperation in the Field of Environmental Research.

EPA Ocean Disposal Bioassay Working Group. 1978. BIOASSAY PROCEDURES FOR THE OCEAN DISPOSAL PERMIT PROGRAM. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. 121 p.

Bioassay procedures are described for toxicity evaluations of waste materials being considered for ocean disposal under EPA's Ocean Disposal Permit Program. Procedures specify use of various organisms representing several trophic levels. Flow-through and static tests are included; methods vary in their utility and complexity. These procedures are not considered "standard methods," but as reference methods or official methods to be used as specified by the EPA Regional Administrator responsible for the permit program. This manual is a revision of EPA-600/9-76-010 published in May 1976.

Evans, John E. 1978. FEASIBILITY OF USING BACTERIAL STRAINS (MUTAGENESIS) TO TEST FOR ENVIRONMENTAL CARCINOGENS. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-78-042. 118 p.

This literature review includes published data on the feasibility of using bacteria as screening agents to detect environmental carcinogens. Mutagenicity data are included because growing experimental evidence indicates that most chemical carcinogens are mutagens, and many mutagens may be carcinogens. This report indicates that bacterial mutagenesis can be used to initiate studies designed to screen for potential mutagens and carcinogens in mixed chemical wastes.

Hansen, D.J. 1978. LABORATORY CULTURE OF SHEEPSHEAD MINNOWS *(CYPRINODON VARIEGATUS)*. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 107-108.

Techniques used at the U.S. EPA Environmental Research Laboratory in Gulf Breeze for the culture of sheepshead minnows in aquaria with under-substrate filters or in aquaria supplied with saltwater are described. The procedure accommodates planning for tests to assure availability of required embryos for life-cycle tests, as well as sufficient juveniles for acute static or flow-through tests after acclimation for 2 weeks.

Hansen, David J. 1978. IMPACT OF PESTICIDES ON THE MARINE ENVIRONMENT. In: First American-Soviet Symposium on the Biological Effects of Pollution on Marine Organisms, Thomas W. Duke and Anatoliy I. Simonov, editors. Environmental Research Laboratory,

Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-007. pp. 126-137.

Effects of toxicants on the entire life cycle of an oviparous estuarine fish, *Cyprinodon variegatus*, can now be studied; preliminary experiments reveal that this fish typically develops from an embryo to maturity in 10 to 14 weeks, with about 70% survival in the laboratory. Females produce an average of eight eggs per day and fertilization success exceeds 90%. Effects of polychlorinated biphenyl, Aroclor® 1254, and of a pesticide, toxaphene, on developing communities of estuarine animals have been investigated. These studies provide data for predicting pollution-induced shifts in composition of estuarine and animal communities.

Hansen, D.J., P.R. Parrish, S.C. Schimmel, and L.R. Goodman. 1978. LIFE-CYCLE TOXICITY TEST USING SHEEPSHEAD MINNOWS *(CYPRINODON VARIEGATUS)*. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 109-117.

The method described determines effects of continuous exposure of a toxic material on sheepshead minnow embryos and fry; their survival and growth to adulthood, and spawning success. Spawning success is measured by the ability of the fish to spawn naturally, number of eggs spawned, fertilization success, and survival of embryos and fry. Experiment requires 4 to 6 months.

Hansen, David J., Steven C. Schimmel, Del Wayne Nimmo, Jack I. Lowe, Patrick R. Parrish, and William H. Peltier. 1978. STATIC METHOD FOR ACUTE TOXICITY TESTS USING FISH AND MACROINVERTEBRATES. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600-9-78-010. pp. 89-96.

Procedures are described for acute toxicity tests with fish in containers 15 to 20 cm in depth. Tests require saltwater in which healthy animals can survive throughout acclimation and testing without stress as evidenced by unusual behavior or discoloration. Appropriate test animals and test materials are specified.

Hansen, David J., Steven C. Schimmel, Del Wayne Nimmo, Jack I. Lowe, Patrick R. Parrish, and William H. Peltier. 1978. FLOW-THROUGH METHODS FOR ACUTE TOXICITY TESTS USING FISH AND MACROINVERTEBRATES. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 97-106.

Continuous-flow (often referred to as flow-through) bioassays are preferred over static tests in evaluating certain types of wastes to be disposed of at sea, particularly those with high biochemical oxygen demands and those that are unstable or volatile. Many test species of fish and macroinvertebrates have high metabolic rates and are difficult to maintain in jars or tanks of standing seawater. A method is described for a 96-hr, flow-through bioassay on marine fish and macroinvertebrates appropriate for the evaluation of wastes.

Jackson, Betty P., editor. 1978. RESEARCH REVIEW. 1977. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-014, 64 p.

This report summarizes results of aquatic research conducted by the Environmental Research Laboratory, Gulf Breeze, FL, office of Research and Development, U.S. Environmental Protection Agency, from January 1 to December 30, 1977. The research program examines the impact of pesticides and other organic compounds on marine species and communities, and seeks to develop new methodology for determining ecological hazards of chemical substances under simulated natural conditions. Projects are outlined under four categories: research related to toxicological testing; biological processes and effects; development of offshore oil resources; and Kepone in the marine environment. Investigations conducted at the laboratory's Atlantic Coast field station at Bears Bluff, SC, also are reviewed for the year 1977.

Jackson, Betty P., editor. 1978. SYMPOSIUM ON PROTECTING THE MARINE ENVIRONMENT: RESEARCH AND REGULATION. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Reporting Series, EPA 600/9-78-006. 38 p.

This symposium focuses on the essential role of research and regulator agencies in protecting marine ecosystems. Purpose of the symposium is to commemorate dedication of a new toxicological test facility at the U.S. Environmental Protection Agency's Environmental Research Laboratory in Gulf Breeze, FL, on October 7, 1977. Participants define the special function of the federal agency scientist, the social responsibility of the scientist, and the need for research in support of environmental regulation. Historical and future objectives of the Gulf Breeze Laboratory are also reviewed.

Jackson, Betty P. and Andree F. Lowry, editors. 1979. PUBLICATIONS GULF BREEZE LABORATORY. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-79-036. 115 p.

This bibliography, inclusive from 1971 through 1978, lists all publications authored by researchers employed by the Environmental Research Laboratory, Gulf Breeze, and its field station on St. Johns Island, SC, or by researchers conducting studies under funding or direction of the laboratory.

Koch, Robert B. 1978. DETERMINATION OF THE SITE(S) OF ACTION OF SELECTED PESTICIDES BY AN ENZYMATIC-IMMUNOBIOLOGICAL APPROACH. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-78-093. 29 p.

This report describes development of an antibody to an organochlorine pesticide to be used in studies related to its inhibition of the ATPase system. Kelevan, the condensation product of ethyl levulinate and Kepone, was successfully conjugated to bovine serum albumin (BSA), fibrinogen (BF), and gamma globulin (BGG). Rabbits and chickens preimmunized with BSA and then immunized with BSA-Kelevan produced antibodies to both the hapten, Kelevan, and the carrier protein BSA. Antiserum to Kelevan protected ATPase activity against Kepone and its derivatives. The titer of antibody to Kelevan was critical since antiserum with only trace amounts of Kelevan antibody failed to protect the ATPase activity against Kepone inhibition. Antibody was concentrated by Na_2SO_4 fractional precipitation of the antiserum and obtained in pure form by affinity chromatography with BGG-Kel covalently linked to Sepharose 4B. Pure antibody was obtained from untreated blood serum or plasma with no prior pretreatment or fractionation with the BGG-Kel affinity column. Complete protection of mitochondrial mg^{2+}ATPase activity from *in vitro* inhibition of Kepone was obtained with a 1.2 mg quantity of Na_2SO_4 fractionated antibody and only 120 µg of pure antibody. Reversal of ATPase inhibition was readily obtained by addition of antibody prior to addition of substrate to the reaction mixture.

Mix, Michael C. 1979. CHEMICAL CARCINOGENS IN BIVALVE MOLLUSKS FROM OREGON ESTUARIES. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-79-034.

The research undertaken involved the use of indigenous populations of bivalve mollusks as monitors for detecting and quantifying environmental benzo[a]pyrene (BaP) in Oregon estuaries. Short-term and long-term studies were conducted in order to establish baseline levels of BaP and to identify seasonal variations in BaP concentrations in shellfish. A presumptive cellular proliferative disorder, though possibly to be neoplastic, was also studied in mussels *(Mytilus edulis)* from Yaquina Bay.

Histological studies revealed that mussels inhabiting polluted environments, and with high BaP body burdens, had an average 6-8% prevalence of the cellular proliferative disorder while those from clean environments and with low or undetectable levels, did not have the disorder. The cellular condition showed a definite seasonal pattern, there was a low prevalence during the summer and fall followed by an increase during the early winter and a peak prevalence occurred in January-February. The atypical, large cells that characterize the disorder in *M. edulis* possess many ultrastructural properties in common with malignant vertebrate cells.

Nimmo, D.R., T.L. Hamaker, and C.A. Sommers. 1978. CULTURING THE MYSID *(MYSIDOPSIS BAHIA)* IN FLOWING SEAWATER OR A STATIC SYSTEM. In: Bioassay Procedures for Ocean Disposal Permit Program. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 59-60.

Methods are described for the culture of the bay mysid *(Mysidopsis babia)* for life-cycle toxicity tests in (1) flowing seawater and (2) a re-circulating aquarium. The mysid is considered a practical organism for toxicological and physiological studies during sensitive stages of development.

Nimmo, D.R., T.L. Hamaker, and C.A. Sommers. 1978. ENTIRE LIFE-CYCLE TOXICITY TEST USING MYSIDS *(MYSIDOPSIS BAHIA)* IN FLOWING WATER. In: Bioassay Procedures for Ocean Disposal Permit Program. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 64-68.

Procedures of a method are outlined for determining effects of continuous exposure of a pollutant on the survival, reproduction, growth, and behavior of a crustacean *(Mysidopsis babia)* throughout a life cycle. The test species can be captured from small shallow ponds fed by saltwater with a small fish net or a 3- to 4-foot push net of small mesh.

Parrish, Patrick R., Elizabeth E. Dyar, Joanna M. Enos, and William G. Wilson. 1978. CHRONIC TOXICITY OF CHLORDANE, TRIFLURALIN, AND PENTACHLOROPHENOL TO SHEEPSHEAD MINNOWS *(CYPRINODON VARIEGATUS)*. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-78-010. 53 p.

Test results are reported of exposures of sheepshead minnows *(Cyprinodon variegatus)* to three chemicals--chlordane, trifluralin, or pentachlorophenol--in flowing, natural seawater to determine acute and chronic (full life-cycle) effects.

Mortality of parental fish exposed to mean measured chlordane concentrations $\geq$ 2.8 µg/ℓ was significantly greater than that of control fish. Hatch of juveniles from embryos of parental fish exposed to $\geq$ 0.8 µg/ℓ was significantly less than hatch of control juveniles. The estimated maximum acceptable toxicant concentration (MATC) was >0.5<0.8 µg/ℓ and the application factor (AF) limits, 0.04-0.06.

Exposure to mean measured trifluralin concentrations $\geq$ 9.6 µg/ℓ significantly decreased growth of parental fish. Fecundity of parental fish exposed to concentrations $\geq$ 4.8 µg/ℓ was significantly less than that of control fish. Survival and growth of second generation fish

were significantly less than the control in concentrations $\geq$ 9.6 µg/ℓ. The estimated MATC was >1.3<4.8 µg/ℓ and the AF limits, 0.007-0.025.
Mortality of parental sheepshead minnows exposed to mean measured pentachlorophenol concentrations >88 µg/ℓ was significantly greater than mortality of control fish. The estimated MATC was >47<88 µg/ℓ and the AF limits, 0.11-0.20.

Roberts, Morris H., Jr., Chae E. Laird, and Jerome E. Illowsky. 1979. EFFECTS OF CHLORINATED SEAWATER ON DECAPOD CRUSTACEANS AND *MULINIA* LARVAE. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-79-031. 110 p.

Eggs and larvae of decapod crustaceans and embryos of *Mulinia lateralis* were exposed to chlorinated seawater for varying periods in continuous flow systems. Mortality, developmental rate, and general behavior were recorded. *Panopeus herbstii* zoeae were more sensitive to chlorine-induced oxidants (CIO) than eggs or adults (96-hr LC50 ca. 2.8 µeq/ℓ = 0.1 mg/ℓ). The 96-hr LC50 for *Pagurus longicarpus* zoeae was approximately the same as for *Panopeus* zoeae. The 120-hr LC50 for *Pagurus* zoeae was 1.4 µeq/ℓ (0.05 mg/ℓ). Development was slightly delayed for *Pagurus* zoeae at CIO levels as low as 0.6 µeq/ℓ (0.02 mg/ℓ). *Mulinia* embryos exposed for 48-hr had an LC50 between 0.3 and 2.8 µeq/ℓ (0.01 and 0.1 mg/ℓ). *Mulinia* embryos exposed to chlorinated seawater for 2 hr had an LC50 of about 2.0 µeq/ℓ (0.072 mg/ℓ); subsequent survival rates for larvae in unchlorinated seawater were unaffected by prior exposure to CIO.
The effects of CIO on serum constituents in *Callinectes sapidus* occurred sporadically and appeared unrelated to dose or mortality. Similar effects were noted for oxygen consumption in whole crabs and excised gills.

Turekian, Karl K., and Anotoliy I. Simonov, editors. 1978. FIRST AMERICAN-SOVIET SYMPOSIUM ON CHEMICAL POLLUTION OF THE MARINE ENVIRONMENT. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-038. 199 p.

This symposium, organized under a U.S.-U.S.S.R. Environmental Agreement (Project 02.06-21), focuses on the impact of chemical pollution on the world's oceans. Soviet and American specialists discuss the fate of heavy metals in estuaries and the Gulf of Mexico; transport of natural radionuclides in shelf waters of the eastern U.S.; the distribution and dynamics of trace metals in pore water and sediment; biogeochemical research on metals in the world's oceans; monitoring chemical pollution and forecasting its biological consequences; arsenic, antimony, and mercury in seawater; pollution of the Caribbean Basin; oil and oil products in surface waters of the Atlantic, Pacific, and Indian Oceans; the forms of heavy metals in seawater (e.g. mercury); methods of sampling water from the ocean surface microlayer and the technical composition of the microlayer; a method for determining mercury; scientific aspects of marine pollution problems; and the management of the quality of the marine environment.

Tyler-Schroeder, Dana Beth. 1978. CULTURE OF THE GRASS SHRIMP *(PALAEMONETES PUGIO)* IN THE LABORATORY. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 69-72.

The grass shrimp *(Palaemonetes pugio)* is useful in assessing toxicity of various materials. It is (1) easily cultured in the laboratory and sensitive to toxicants, and (2) can be exposed to toxicants in flow-through aquaria throughout its life cycle. Culture and holding procedures are described.

Tyler-Schroeder, D.B. 1978. STATIC BIOASSAY PROCEDURE USING GRASS SHRIMP *(PALAEMONETES* SP.) LARVAE. In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 73-82.

Procedures are outlined for static 96-hr bioassays with the grass shrimp larvae, *Palaemonetes* sp. Three species of the genus, *P. pugio, vulgaris,* and *intermedius,* are easily collected in the field and maintained in the laboratory. Spawning can be induced in the laboratory by manipulating temperature and light. Developing larvae have demonstrated a greater susceptibility to polychlorinated hydrocarbons than observed in adults or juveniles.

Tyler-Schroeder, Dana Beth. 1978. ENTIRE LIFE-CYCLE TOXICITY TEST USING GRASS SHRIMP *(PALAEMONETES PUGIO* HOLTHUIS). In: Bioassay Procedures for Ocean Disposal Permit Program, Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-010. pp. 83-88.

A method to assess toxicity of a material to all life stages of the grass shrimp in flow-through systems is described. Tests are conducted throughout the life cycle of the shrimp--from juvenile stage of the parental generation, sexual maturation and reproduction, through hatching, larval development and growth of the F_1 generation to juvenile stage. Thereafter, tests may terminate, or exposures can be continued if a determination of effects on F_1 reproduction and F_2 larval development is required.

Vernberg, F.J., W. Kitchens, H. McKellar, K. Summers, and R. Bonnell. 1978. THE DYNAMICS OF AN ESTUARY AS A NATURAL ECOSYSTEM, VOL. II. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-78-092. 29 p.

This report describes two separate but interrelated substudies: an update of the macroecosystem model of

the North Inlet Estuary near Georgetown, SC, and a continuing study of experimental saltmarsh microecosystems. The model is under development to help understand the interactions of various parts of a natural ecosystem. The principal objective of the study is to develop and test replicate experimental salt-marsh units at the microecosystem level as diagnostic tools for assessing long- and short-term pollution effects on the *Spartina alterniflora* salt-marsh community. Because of the complexity, this study was conceived as a five-year work. Two years of study (March 1, 1976, to February 28, 1978) are reported. A summary of the first phase of this research is contained in the Ecological Research Series (EPA-600/3-77-016, January 1977).

Walker, William W. 1978. INSECTICIDE PERSISTENCE IN NATURAL SEAWATER AS AFFECTED BY SALINITY, TEMPERATURE, AND STERILITY. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/3-78-044. 25 p.

Effects of temperature, salinity, and sterility on the degradation of malathion, parathion, methyl parathion, diazinon, and methoxychlor in fresh and estuarine water under controlled conditions are reported. Surface water samples of 1, 10, 20, and 28 °/oo salinity were amended with these insecticides and incubated in the dark at 30°, 20°, and 10°C under sterile and nonsterile conditions. Insecticide abatement was followed by electron-capture gas-liquid chromatographic techniques.

No significant differences between sterile and nonsterile treatments were observed for any of the insecticide studies; the effect of increasing temperature was highly significant with regard to increased degradation of malathion, parathion, methyl parathion, and diazinon. Methoxychlor reflected the recalcitrance characteristic of the chlorinated hydrocarbon insecticides throughout 84 days of incubation and was not significantly affected by salinity, temperature, or sterility. Salinity effects were varied among the four organophosphates: highly significant for malathion and diazinon, significant for methyl parathion, and not significant for parathion.

Wilkes, Frank G. 1978. MICROCOSMS AS BIOLOGICAL INDICATORS OF POLLUTION. In: First American-Soviet Symposium on the Biological Effects of Pollution on Marine Organisms, Thomas W. Duke and Anatoliy I. Simonov, editors. Environmental Research Laboratory, Gulf Breeze, FL. U.S. EPA Ecological Research Series, EPA-600/9-78-007. pp. 155-56.

Research conducted and supported by the Environmental Research Laboratory, Gulf Breeze, to develop microcosms as a method for investigating pollutant fate and effects in the environment is described. Ecosystem compartments under investigation include direct accumulation from water and food by organisms at all trophic levels, bioaccumulation through food chains, direct effects of pollutants on organisms, i.e., mortality, reproduction and behavior, and indirect effects of sublethal levels of pollutants, such as changes in predator-prey relationships. Microbial processes at both air-water and sediment-water interfaces are investigated as well as physical and chemical transformations.

Journal Articles

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Food Chain Bioassay	L.H. Bahner
Algal Stimulation/Inhibition Statistical Model	L.H. Bahner G.E. Walsh
Effects of Toxicants (Flora and Fauna)	G.E. Walsh
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